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A comparison between two structurally defined chlorobiphenyls — 2,4′,5-trichlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl — with regard to biological effects and behaviour in the animal body

Jan Örberg

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Solna, January, 1978

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Research Secretariat

A comparison between two structurally defined chlorobiphenyls - 2,4',5 - trichlorobiphenyl and 2,2',4,4',5,5'- hexachlorobiphenyl - with regard to biological effects and behaviour in the animal body.

by

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Fil. kand., Skånel.

Doctoral dissertation

to be publicly discussed in the lecture hall of the Institute of Zoophysiology, University of Uppsala on March 10, 1978 at 9.00 a.m. for the Degree of Doctor of Philosophy (according to the Royal proclamation No. 327, 1969).

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Errata

1. 28 Referensen Koeman et al. (1969) står Noe ver — skall vara Noever

1. 35 Tabell 1 utgår och ersätts av följande tabell:

Table 1. Hepatic cytochrome P-450 content in female mice after oral administration for 4 consecutive days of 2,4′,5 - trichlorobiphenyl (1.5 mg in 0.2 ml peanut oil/day, groups T_8 and T_{29}) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (1.5 mg in 0.2 ml peanut oil/day, groups H_8 and H_{29}) or peanut oil (0.2 ml/day, groups C_8 and C_{29}) compared by means of t-test. The t-values are corrected for small samples according to de V. Weir (1960)[x]

Group	No. of animals	Day of analysis [xx]	Cytochrome P-450 n mol/mg microsomal protein Mean ± S.D.
C_8	6	8	0.78 ± 0.14
T_8	6	8	1.17 ± 0.18 [a]
H_8	6	8	1.47 ± 0.15 [a,b]
C_{29}	5	29	0.78 ± 0.02
T_{29}	6	29	0.74 ± 0.14 [d]
H_{29}	6	29	0.94 ± 0.15 [c]

[x] de V. Weir, J.B.: Standardized t. Nature (Lond.) 1960, 185, 558.

[xx] The day when the oral administration of the drug started was designated day 1.

a) Significantly different from C_8 ($p < 0.0025$)
b) Significantly different from T_8 ($p < 0.01$)
c) Significantly different from C_{29}, T_{29} ($p < 0.025$)
d) Significantly different from T_8 ($p < 0.0025$)
e) Significantly different from H_8 ($p < 0.0005$)

1. 35 Rad 11 nedifrån står Brickers — skall vara Bickers

1. 38 Rad 6 står Brickers — skall vara Bickers

1. 38 Följande referenser utgår:
Carlson & Ruby (1973), Keplinger et al. (1971) och Stalling & Mayer (1972).

1. 40 Rad 14 nedifrån står (unpublished results) — skall vara (1977).
(Örberg, J.: Placental and mammary transfer of two PCBs (2,4′,5 - TCB and 2,2′,4,4′,5,5′- HCB) and their effect on the reproductive capacity in mice. Ambio 1977, 5, 278-280.

. 60 Table 2.

Står		— skall vara	
	Liver (mother) ppm TCB or HCB Mean S ± D		Liver (mother) ppm TCB or HCB Mean ± SD

A comparison between two structurally defined chlorobiphenyls - 2,4',5 - trichlorobiphenyl and 2,2',4,4',5,5' - hexachlorobiphenyl - with regard to biological effects and behaviour in the animal body.

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This thesis is based on the following papers:

I Örberg, J.: A comparison between some effects of two pure chlorobiphenyls (2,4′,5 - trichlorobiphenyl and 2,2′,4,4′,5,5′- hexachlorobiphenyl) on the hepatic drug metabolizing system in the female mouse. Acta pharmacol. toxicol. 1976, 39, 145-151.

II Örberg, J. & C. Ingvast: Effects of pure chlorobiphenyls (2,4′,5 - trichlorobiphenyl or 2,2′,4,4′,5,5′- hexachlorobiphenyl) on the disappearance of ^{14}C from the blood plasma after intravenous injection of 4-^{14}C-progesterone and on the hepatic drug metabolizing system in the female rat. Acta pharmacol. toxicol. 1977, 41, 11-17.

III Örberg, J.: Effects of pure chlorobiphenyls (2,4′,5 - trichlorobiphenyl and 2,2′,4,4′,5,5′- hexachlorobiphenyl) on the reproductive capacity in female mice. Acta pharmacol. toxicol. Accepted for publication.

IV Örberg, J.: Placental and mammary transfer of two PCBs (2,4′,5 - TCB and 2,2′,4,4′,5,5′- HCB) and their effect on the reproductive capacity in mice. Ambio 1977, 5, 278-280.

V Örberg, J.: Effects of pure chlorobiphenyls (2,4′,5 - trichlorobiphenyl and 2,2′,4,4′,5,5′- hexachlorobiphenyl) on the post-natal growth in mice. Acta pharmacol. toxicol. Accepted for publication.

The papers will be referred to by the Roman numerals given above.

1. INTRODUCTION.

Polychlorinated biphenyls (PCBs) constitute a large group of closely related substances. They differ from one another with regard both to the number and the positions of the chlorine substituents. Due to the chemical and physical properties of the PCBs - high dielectric constants, chemical and thermal stability - they are very attractive for industrial use. PCBs occur e.g. in electric equipment (as insulating material), in paints (to increase the stability against chemicals and oxidation) and in rubbers and synthetic resins (as plasticizers).

Industrially the PCBs are prepared by chlorination of biphenyl (Hubbard 1964). This procedure results in complex mixtures of different homologs and isomers of chlorobiphenyls. Although the commercial production on a large scale of polychlorinated biphenyls started already in 1929 (Hubbard 1964), these substances were not discovered in biological materials until 1966 (Jensen 1966). After this discovery the presence of PCBs has been reported in animals and plants from all over the world (for review see Peakall 1975). As most chlorobiphenyls, especially those with a moderate or a high chlorine content are very resistant to biological degradation they are retained in the organisms and accumulated up the food chains. Consequently animals at the top of the chains e.g. birds of prey and seals contain large amounts of these compounds (for review see Peakall 1975).

Biological effects of chlorinated biphenyls were first studied by Smyth (1931), who found that monochlorobiphenyls given per os to guinea-pigs and rabbits were slightly toxic whereas a mixture of polychlorinated biphenyls under similar conditions proved to be non-toxic. In contrast to that Drinker et al. (1937) and Bennett et al. (1938) presented results from experiments on rats showing toxic effects of PCBs.

As the interest in biological effects of PCBs among research workers was low during the forties and fifties our knowledge in this

field did not increase very much during these decades. However, results from numerous investigations performed during the last 10 - 12 years have given us valuable information about the polychlorinated biphenyls concerning their biological effects, their behaviour in biological systems and their concentrations in organisms from most parts of the world. In brief, some of the results from these investigations have shown that (for review see Peakall 1975):

- the acute toxicity of PCBs is low (Bruckner et al. 1973, Grant & Phillips 1974),
- PCBs are potent inducers of the hepatic drug metabolizing system (Street et al. 1969, Litterst et al. 1972, Johnstone et al. 1974),
- the breakdown of sex steroids e.g. estradiol and testosterone is increased in liver microsomes from birds pretreated with PCBs (Risebrough et al. 1968, Lincer & Peakall 1970, Nowicki & Norman 1972),
- the reproductive capacity is decreased in mammals exposed to PCBs (Ringer et al. 1972, Linder et al. 1974, Kihlström et al. 1975, Jonsson Jr. et al. 1975/1976, Helle et al. 1976, Jensen et al. 1977),
- the immune response is depressed in animals exposed to PCBs (Friend & Trainer 1970, Vos & de Roij 1972),
- PCBs are accumulated in certain steroid producing organs (e.g. adrenals, corpora lutea) (Melvås & Brandt 1973, Brandt 1977).
- PCBs are transferred from females to fetuses and to sucklings via the placenta and via the milk, respectively (Westöö et al. 1970, Platonow et al. 1971, Grant et al. 1971 a, Melvås & Brandt 1973, Brandt 1977).

It was believed for a long time that the animal body was unable to metabolize polychlorinated biphenyls. However, results presented by

Koeman et al. (1969) and Grant et al. (1971 b) showed that biphenyl components with a low chlorine content appearing in commercial PCB preparations are metabolized by quails and rats. This suggested that biphenyls with a low chlorine content might turn out to be less hazardous to the environment than biphenyls with a moderate or a high chlorine content. This idea was supported by results showing that PCB preparations with a low chlorine content are less potent inducers of the hepatic drug metabolizing system than those with a moderate or a high chlorine content (Bickers et al. 1972, Litterst et al. 1972, Chen & DuBois 1973).

If there really are significant differences between biological effects caused by different types of PCB preparations it would be wise to restrict the industrial use to the least dangerous PCB preparations. This is probably a better way to protect the environment than a prohibition against all use of PCBs. - Such a prohibition would probably result in an introduction of substitutional chemicals which may be more harmful to the environment than the PCBs themselves. In any case, a reliable evaluation of the hazards connected with the use of the substitutional chemicals would require a considerable amount of research resources and could not be performed until several years after the introduction of these chemicals.

The purpose of the investigations summarized in this thesis was to compare two chlorobiphenyls - the one with a low chlorine content - - 2,4′,5 - trichlorobiphenyl and the other with a moderate chlorine content - 2,2′,4,4′,5,5′- hexachlorobiphenyl - with regard both to their biological effects and to their behaviour in the animal body.

2. EXPERIMENTAL

Animals: The animals used in the experiments were NMRI-mice and Sprague-Dawley rats (Anticimex, Sweden).

Chemicals: The chlorobiphenyls studied - 2,4',5 - trichlorobiphenyl (henceforth called TCB) and 2,2',4,4',5,5'- hexachlorobiphenyl (henceforth called HCB) - were synthesized by Drs C.A. Wachtmeister, G. Sundström and Å. Bergman, Wallenberg Laboratory, University of Stockholm, Stockholm, Sweden.

Methods: The methods used in the different experiments are presented in detail in the respective papers.

3. RESULTS AND DISCUSSION.

3.1 Disappearance from the liver.

In female mice given a single dose of a peanut oil solution containing equal parts (weight basis) of TCB and HCB by oral administration, the disappearance from the liver of TCB was much faster than that of HCB (I). TCB and HCB both reached their maximum concentrations in the liver about 1.5 hours after the administration. 22.5 hours later (24 hours after the chlorobiphenyl administration) the concentration in the liver of TCB was only about 3 per cent of its maximum concentration whereas the corresponding figure for HCB was nearly 40 per cent (I).

These results are in agreement with results from investigations concerning the ability of the animal body to metabolize chlorinated biphenyls. Various authors have shown that biphenyls with a low chlorine content are more rapidly metabolized than those with a high chlorine content (Koeman et al. 1969, Grant et al. 1971b, Hutzinger et al. 1972, de Freitas & Norstrom 1974).

Brandt (1977) found by means of autoradiography that the elimination from the body of ^{14}C-TCB is rapid in mice. - The highest concentration of radioactivity in the body a few minutes after the injection (i.v.) of ^{14}C-TCB was found in the small intestine. In contrast to ^{14}C-TCB the elimination of ^{14}C-HCB proved to be very slow.

These findings of Brandt (1977) indicate that the decline of the concentration in the liver of HCB (I) mainly is a result of a redistribution of HCB from the liver to the body fat rather than a result of an elimination from the body, whereas the corresponding decline of TCB (I) mainly reflects an elimination of TCB from the body.

3.2 Placental and mammary transfer.

In pregnant and nursing mice exposed to either TCB or HCB during the period of gestation and the period of lactation, respectively, significant amounts of chlorobiphenyls were transferred from the females to the fetuses and to the sucklings (IV). The chlorobiphenyl levels in fetuses (liver) and sucklings (liver and content of the stomach) of females given low doses of HCB (0.05 mg/day) were about ten times higher than those in fetuses and sucklings of females given the same doses of TCB (IV).

This does not necessarily mean that the HCB molecule has physical and chemical properties significantly more favourable to placental and mammary transfer than the TCB molecule. As chlorobiphenyls with a low chlorine content are more easily metabolized than biphenyls with a moderate chlorine content (Koeman et al. 1969, Grant et al. 1971b, Hutzinger et al. 1972, de Freitas & Norstrom 1974), the average level of chlorobiphenyl is likely to be much higher in animals exposed to HCB than in animals exposed to TCB. In this study (IV) the concentrations of chlorobiphenyls in the livers of the mother animals were 10 - 12 times higher in females given HCB than in females given TCB. Thus, it seems most probable that the chlorobiphenyl levels in fetuses and milk

merely reflect the levels of the mother animals. This may also explain the results from experiments on pregnant rabbits given either Aroclor 1221 (containing 21 per cent chlorine) or Aroclor 1254 (containing 54 per cent chlorine). In these experiments Grant et al. (1971 a) found that the levels in fetal livers of Aroclor 1254 were much higher than those of Aroclor 1221.

3.3 Effects on the liver function.

Effects of the two chlorobiphenyls (TCB and HCB) on the hepatic drug metabolizing system in mice and rats have been studied by determining the hepatic content of cytochrome P-450, assessed by the ratio in the microsomal fraction between the amount of cytochrome P-450 and the total amount of proteins (I, II, III) (Cytochrome P-450, which is a heme protein located in the endoplasmatic reticulum, plays a key role in oxidations of drugs.), the liver somatic index (LSI, - the liver weight expressed as per cent of the total body weight) (I, II, III), the hexobarbital induced sleeping time (I) and the disappearance from the blood of ^{14}C after an intravenous injection of 4- ^{14}C-progesterone (II).

3.3.1 Experiments on mice.

In the experiments described in paper I virgin female mice were given either TCB or HCB - 1.5 mg/day for four consecutive days. The females were killed and the hepatic content of cytochrome P-450 determined either Day 8 or Day 29 (Day 1 = the first day of chlorobiphenyl administration). The results obtained showed that experimental females, killed Day 8 , had significantly higher contents of hepatic cytochrome P-450 than the controls and that females exposed to HCB also had significantly higher cytochrome P-450 values than females exposed to TCB. During the period from Day 8 to Day 29 a significant

reduction of the hepatic content of cytochrome P-450 took place in the experimental females. The results from females killed Day 29 showed that there was still a difference in hepatic cytochrome P-450 content between females given HCB and control females, and also between females given HCB and those given TCB, whereas there was no difference between females given TCB and control females.

In addition to effects of the two chlorobiphenyls on the hepatic content of cytochrome P-450 also effects on the LSI and on the hexobarbital induced sleeping time were found. The liver weight was higher and the breakdown of hexobarbital was faster in animals given either of the two chlorobiphenyls than in control animals. These effects were more pronounced in animals given HCB than in animals given TCB. Thus, under these experimental conditions HCB proved to be a more potent inducer of the hepatic drug metabolizing system than TCB.

In the experiments described in paper III mated female mice, divided into four groups, were given 0.05 mg/day or 0.50 mg/day of either TCB or HCB, respectively for three consecutive days, starting on the first day of pregnancy. The day after the last chlorobiphenyl administration the females were killed and the hepatic content of cytochrome P-450 determined. The results obtained under these conditions showed that the hepatic contents of cytochrome P-450 of females given either HCB (0.05 mg/day or 0.50 mg/day, respectively) or TCB (0.50 mg/day) were significantly higher than those of control females. No significant difference in hepatic content of cytochrome P-450 was obtained either between females given 0.05 mg TCB/day and control females or between females given HCB and those given the corresponding amount of TCB. Thus, in contrast to the results presented in paper I, under these experimental conditions HCB did not turn out to be a more potent inducer of the hepatic drug metabolizing system than TCB.

In these two studies (I and III) the hormonal status of the females, which has been shown to affect the activity of the drug metabolizing system (Noordhoek 1972), probably differed. However, it does not seem very probable that a change in the hormonal status would alter the relation between TCB and HCB with regard to their ability to stimulate the drug metabolizing system. Moreover, the amounts of the chlorobiphenyls given to the females and the time elapsed from the chlorobiphenyl administration to the killing of the females differed between the experiments.

The results presented in paper III showed that the hepatic cytochrome P-450 values obtained from females killed the day after their last chlorobiphenyl administration were lower, but not significantly lower in females given TCB than in females given HCB. This indicates that there is no drastic difference between TCB and HCB as to their ability to increase the hepatic content of cytochrome P-450. The results presented in paper I showed that effects on the drug metabolizing system caused by HCB were more persistent than those caused by TCB. This difference is probably due to the fact that TCB disappears from the liver much faster than HCB (I).

3.3.2 Experiments on rats.

Results from experiments on sexually mature, virgin female rats given either HCB or TCB, 0.5 mg/day for 14 consecutive days and killed the day after the last chlorobiphenyl administration, showed that the hepatic content of cytochrome P-450 was significantly higher in females exposed to HCB than in control females and in females exposed to TCB (II). However, the hepatic cytochrome P-450 values obtained from control females and from females given TCB did not differ from one another (II). The disappearance of ^{14}C (originating from an intravenous injection of 4-^{14}C-progesterone, given the day after the last chlorobiphenyl administration) from the blood plasma was significantly faster in females given HCB than in

control females whereas there was no significant difference in this respect between females given TCB and control females (II).

Kuntzman et al. (1964) have shown that steroid hormones e.g. estradiol and testosterone are normal substrates for the drug metabolizing enzymes. As female rats pretreated with HCB have a high activity in the drug metabolizing system (II) it is obvious that the rapid disappearance of ^{14}C from the blood in these animals is caused by an increased breakdown of progesterone. It is known that the metabolism, in vitro, of sex steroids is increased in liver microsomes from birds pretreated with PCBs (Risebrough et al. 1968, Lincer & Peakall 1970, Nowicki & Norman 1972). Örberg & Lundberg (1974) have also reported results indicating an enhanced breakdown, in vivo, of testosterone in male mice exposed to PCBs.

Under the experimental conditions used in the experiments described in papers II and III low doses of TCB had no detectable effect on the hepatic drug metabolizing system either in the female rat or in the female mouse. As mentioned before, in female mice, TCB disappears much faster from the liver than HCB (I). The level of chlorobiphenyl in livers from female rats exposed to TCB was much lower than in livers from females exposed to HCB (II). This suggests that also in the rat the disappearance from the liver of TCB is much faster than that of HCB. Thus, in animals given low doses of TCB once a day, the amounts of TCB entering the liver disappears rapidly and the average concentration of TCB in the liver cells is apparently kept below that causing effects on the drug metabolizing system, detectable by means of the methods used.

The results summarized in this paragraph are in agreement with results from other investigations showing that effects of chlorinated biphenyls on the hepatic drug metabolizing system are less pronounced if the biphenyl has a low chlorine content than if it has a moderate or a high chlorine content (Bickers et al. 1972, Litterst et al. 1972, Chen & Du Bois 1973, Johnstone et al. 1974). However, the stimulating effects

of a chlorobiphenyl on the hepatic drug metabolizing system are not only dependent upon its chlorine content but apparently also upon the positions of its chlorine substituents. From experiments on rats given isomers of different chlorobiphenyls Ecobichon & Comeau (1975) obtained results suggesting that chlorine substituents in 4,4´-positions increase the ability of the chlorobiphenyl to induce microsomal enzymes in the liver.

3.4 Effects on the reproductive capacity.

Effects of the two chlorobiphenyls (TCB and HCB) on the reproductive capacity in mice have been studied under two different experimental conditions.

In experiments described in paper IV both sexes were exposed to either TCB or HCB. The exposure started already before birth and ended at weaning, when the young were 21 days old. During this period the pregnant/nursing females were given either TCB or HCB (0.05 mg/day). No chlorobiphenyl was given directly to the fetuses or to the sucklings. Their only sources of chlorobiphenyls were the maternal blood (during the gestation) and the milk (during the lactation). After reaching sexual maturity the animals were placed in cages in pairs. The females were killed in the middle of gestation and the number of fetuses were counted. The results obtained from either of the two experimental groups and from the control group did not differ significantly from one another. Thus, neither the number of fetuses per mated female, the number of fetuses per pregnant female nor the frequency of pregnancies did differ between the three different groups.

In experiments described in paper III adult female mice were exposed to either TCB or HCB. The exposure started on the first day of pregnancy (the day when a vaginal plug was found) and ended five days later. During this period the females were given 0.05 mg/day or

0.50 mg/day of either TCB or HCB, respectively. They were killed the day after the last chlorobiphenyl administration and the number of corpora lutea and placentae were counted, considering each placenta as indicating an implantation. The results from this study showed that females given the high dose (0.50 mg/day) - but not those given the low dose (0.05 mg/day) of TCB or HCB had significantly lower frequencies of implanted ova x) than the control females. However, no differences could be demonstrated between the five different groups (four experimental groups and one control group) in either the number of implanted ova per pregnant female or the frequency of pregnancies. Thus, no significant difference between the effects of the two chlorobiphenyls on the reproductive capacity was found.

The implantation of the blastocyst is controlled by ovarian hormones (progesterone and estrogen) (for review see Nalbandov 1971). The role of these hormones in implantation differs between species. For instance, in rabbits implantation can be induced by progesterone alone (Chambon 1949) but in mated ovariectomized mice given daily injections of progesterone no implantation occurs unless a single dose of estrogen is administered (Smith & Biggers 1968). There are also results showing that the number of implanted ova increases with increasing doses of estrogen (within certain limits) (Smith & Biggers 1968).

In mated mice and rats there is a peak in the plasma level of estradiol on the day before the implantation (Shaikh 1971, McCormack & Greenwald 1974). This preimplantation rise in plasma level of estradiol is believed to be the trigger of the implantation process in these species.

When combining these facts with those concerning effects of PCBs on the metabolism of steroid hormones (Risebrough et al. 1968, Lincer & Peakall 1970, Nowicki & Norman 1972) it is tempting to adopt the current hypothesis that the decreased reproductive capacity in mammals exposed to PCBs is caused by subnormal levels of steroid

x) (frequency of implanted ova = the ratio between the number of implanted ova and the number of shed ova).

hormones as a result of a PCB induced increase of the breakdown of these hormones. If this hypothesis is correct it means that there is a negative correlation between the activity in the drug metabolizing system and the frequency of implanted ova.

This hypothesis is supported by results presented in paper III. - Females given the high dose (0.50 mg/day) of TCB or HCB, respectively, exhibit low frequencies of implanted ova and a high activity in the hepatic drug metabolizing system at the time of the preimplantation rise in the plasma estradiol level. The unexpected result that the frequency of implanted ova does not differ between females from these two groups may be explained by means of this hypothesis as well. - As the effects of the two chlorobiphenyls on the hepatic content of cytochrome P-450 in these females do not differ significantly from one another there are reasons to believe that the effects of the two chlorobiphenyls on the metabolism of sex steroids do not either. This means that the reason for the two chlorobiphenyls to produce different effects on the implantation process does not exist.

This hypothesis assumes that the animal body is unable to compensate an increased breakdown of sex steroids by an increased synthesis of these hormones. However, experimental findings presented during the last years have provided information making it necessary to discuss alternative explanations to the effects of PCBs on the reproductive capacity in mammals. Results presented by Örberg & Lundberg (1974) strongly indicate that male mice exposed to PCBs increase the synthesis of testosterone. This compensatory synthesis, probably stimulated via the gonadal-hypophyseal feed back mechanism, restore the plasma levels of testosterone to normal within 28 days. Unpublished results from experiments on mink bitches have shown that the plasma levels of progesterone in pregnant bitches exposed to PCBs do not differ from those in pregnant control bitches. (The hepatic cytochrome P-450 content was significantly higher in bitches exposed to PCBs than in control bitches.) This indicates that

also mink bitches are able to compensate an increased steroid breakdown by an increased synthesis. There are no reasons to believe that mice and minks are unique in this respect. Probably males and females from most mammalian species have this ability. Thus, by means of the hypothesis presented above it is not possible to explain the observed decrease in the reproductive capacity among animals more or less permanently exposed to PCBs (Jonsson Jr. et al. 1975/1976, Helle et al. 1976, Jensen et al. 1977).

In a series of experiments Brandt (1977, personal communication) has studied the tissue localization of different ^{14}C-labelled chlorobiphenyls in mice by means of autoradiography. The results from experiments on pregnant mice have shown that chlorobiphenyls and/or metabolites of chlorobiphenyls are accumulated for instance in the yolk sac epithelium, in the uterine fluid, in the pituitary and in steroid producing organs such as the ovary, the testis and the adrenal cortex. The presence of foreign compounds in the environment of the fetuses may interfere with the normal processes going on in the uterus, possibly resulting in embryonic and fetal death.

Sows in late gestation, exposed to another chlorinated hydrocarbon - lindan (dissolved in petroleum ether), exhibit a high frequency of dead fetuses (Ballsrud personal communication). In these animals the residue levels of lindan were very low in all tissues analysed except for the fetal membranes. From these facts alone it is not possible to determine the cause of the fetal death.

The presence of chlorobiphenyls and/or metabolites of chlorobiphenyls in endocrine organs such as the pituitary, the ovary and the testis may disturb the synthesis and the secretion of gonadotrophins and sex steroids. Results supporting this idea have recently been reported. Female rats exposed to PCBs for 36 weeks exhibit reduced number of follicles, histological changes in the ovarian stromal cells and low plasma levels of progesterone (Jonsson Jr. et al. 1975/1976).

Our knowledge about the physiological effects of PCBs, obtained mainly from laboratory experiments, is insufficient to explain the

causes of the decreased reproductive capacity in mammals exposed to these substances. Under ecological conditions there are animals e.g. seals from the Baltic, which are permanently exposed to significant amounts of PCBs (Olsson et al. 1975). It is most probable that PCBs under such conditions give rise to unknown effects. However, the detrimental effect of PCBs on the reproductive capacity can probably not be ascribed to a PCB-induced change in a single organ or in a single process. It seems more probable to assume that the decreased reproductive capacity is the result of changes in many organs and processes, directly and/or indirectly caused by PCBs.

3.5 Effects on growth.

Chlorobiphenyls given to female mice during the periods of gestation and lactation (0.05 mg/day of either TCB or HCB given from Day five of pregnancy to Day 22 post partum) affect the growth of the fetuses and of the young (V). The birth weights of males born by mothers given HCB were significantly higher than those of males born by control mothers or by mothers given TCB (V). The growth rates during the period of lactation did not differ significantly between the young from the different groups. However, after weaning the growth rates of male young nursed by mothers given either TCB or HCB were significantly higher than those of male young nursed by control mothers, and female young of mothers given TCB exhibited significantly higher growth rates than those of control mothers or of mothers given HCB (V).

Certain steroid hormones from the adrenal cortex - the glucocorticoids, e.g. cortisol and corticosterone - have growth retarding effects (Gaunt 1954). Thus, decreased plasma levels of these hormones may result in increased growth. Such a reduction of the plasma levels of glucocorticoids could be caused by an increased breakdown or/and a decreased synthesis of these hormones.

The breakdown of steroid hormones is increased in animals exposed to chlorinated biphenyls as a result of an enhanced activity in the hepatic drug metabolizing system (Kuntzman et al. 1964, Risebrough et al. 1968, Inao 1970). However, as TCB is a less potent inducer of the drug metabolizing system than HCB (see paragraph 3.3) the breakdown of glucocorticoids is probably not faster in females exposed to TCB than in females exposed to HCB. The synthesis (in vitro) of corticosterone is reduced in the adrenal cortex from rats given PCBs (Inao 1970). The fact that some pure chlorobiphenyls are accumulated in the adrenal cortex (Brandt 1977) may indicate that the hypofunction of the adrenal cortex in these animals is due to a direct effect of PCBs on the function of this organ. However, in contrast to HCB, TCB is not accumulated in the adrenal cortex (Brandt 1977). Apparently, there are no reasons to assume that the plasma levels of glucocorticoids are lower in animals exposed to TCB than in animals exposed to HCB. However, female young of mothers given TCB exhibited growth rates significantly higher than those of mothers given HCB (V). Thus, subnormal levels of glucocorticoids are not likely to be the explanation to the growth promoting effect of the chlorobiphenyls.

Results from other investigations have shown that commercial PCB preparations increase the post natal growth rate in pigs and mice whereas they decrease it in minks and rhesus monkeys (Ringer et al. 1972, Kihlström et al. 1974, Allen & Barsotti 1976, Platonow et al. 1976). This should not be taken as showing that PCBs act as growth promoting substances in some species and as growth retarding substances in other species. Probably, PCBs in suitable dosage have the ability to increase growth in most species and whether they will increase or decrease the growth rate of animals from a given species is likely to be a matter of amounts of PCBs given in relation to the sensitivity of the animals to PCBs. Rather low doses of PCBs given to minks and rhesus monkeys do not only cause retarded growth but also severe pathological anomalies

(Platonow & Karstad 1973, Allen & Barsotti 1976). Therefore it is most likely that the low growth rate in these animals is a result of an impairment of their state of health. Quite high doses of PCBs, - much higher than those causing pathological anomalies in minks and rhesus monkeys, - given to piglets increase the growth rate but do not cause any detectable alterations of the general conditions of these animals (Platonow et al. 1976).

When trying to explain the increased growth in piglets given PCBs, the authors suggested that the anabolic effect of androgens may be involved (Platonow et al. 1976). Results strongly supporting this idea are presented in table 1.

Table 1. Body weights, testis weights and plasma levels of testosterone in growing piglets exposed to PCBs (Aroclor 1254) for one, three, six or 12 weeks, respectively. (Based on results presented by Platonow et al. 1976.)

Time of exposure	1 week		3 weeks		6 weeks		12 weeks	
Animals	Controls	Exposed to PCB	Controls	Exposed to PCB	Controls	Exposed to PCB	Controls	Exposed to PCB
Body weight (kg)	14.6	15.1	20.9	17.9	26.1	35.6	54.2	78.8
Testis weight (g)	9.2	8.9	17.0	14.0	25.7	25.9	43.0	155.2
Plasma testosterone level (ng/ml)	-	-	0.57	0.57	0.14	2.50	0.46	2.51

As seen from this table, piglets given PCBs for six weeks had higher plasma levels of testosterone than control piglets and piglets given PCBs for 12 weeks were heavier, had heavier testes and higher plasma levels of testosterone than control piglets. The plasma levels of testosterone in piglets given PCB for six or 12 weeks, respectively, are comparable to those in boars (Liptrap & Raeside 1975).

Obviously, the testes started to produce large amounts of testosterone much earlier in piglets exposed to PCBs than in control piglets. In other words, in piglets given PCBs the onset of puberty occurred

earlier than in control piglets. The mode and site of action of this effect of PCB is not known.

It cannot be excluded that the high growth rates, after weaning, among mice born and nursed by mothers exposed to chlorobiphenyls (IV) also are due to elevated plasma levels of androgens.

It is interesting to note that not only the postnatal growth but apparently also the prenatal growth of mice are affected by chlorobiphenyls. - Males born by mothers exposed to HCB were significantly heavier at birth than males born by control mothers (V). There are results showing that also the body composition of newborn mice of mothers given HCB differs from that of newborn mice of control mothers (Örberg & Ljungström, to be published). The differences in body weight and in body composition between newborn mice of mothers given HCB and those of control mothers exhibit certain resemblances with the corresponding differences between babies of diabetic women and babies of healthy women (Baird 1969). The body weight and the fat content are higher and the water content is lower in neonates of female mice given HCB and in babies of diabetic women than in neonates of control females and in babies of healthy women, respectively (Baird 1969, Örberg & Ljungström, to be published). The physiological reasons for these effects of HCB are not known but the results presented above indicate a disturbed metabolism of carbohydrates in pregnant mice exposed to HCB.

4. GENERAL DISCUSSION

As indicated in the introduction, the purpose of the investigations summarized in this thesis was to obtain information about differences, if any, between chlorobiphenyls with a low chlorine content and chlorobiphenyls with a moderate chlorine content, with regard both to their biological effects and to their behaviour in the animal body. This was in order to increase our possibilities to assess if a restriction of the industrial use of PCBs, to such PCB preparations containing only biphenyls with a low chlorine content would significantly decrease the biological hazards connected with the use of PCBs.

The results obtained have shown the existence of differences between HCB and TCB both in their biological effects and in their behaviour in the animal body (see table 2).

Table 2. A comparison between HCB and TCB with regard to biological effects and behaviour in the animal body.

Disappearance from the liver	The disappearance from the liver of HCB is much slower than that of TCB (I).
Concentration in milk and tissues (liver, fetus)	The chlorobiphenyl levels are high in milk and tissues from animals exposed to HCB and ten to two hundred times higher than in milk and in the corresponding tissue from animals exposed to TCB (II, III, IV).
Effects on the hepatic drug metabolizing system	The two chlorobiphenyls both have the ability to induce the hepatic drug metabolizing system (I, II, III). To increase the hepatic content of cytochrome P-450 smaller doses of HCB than of TCB is needed (II, III). The effects on the drug metabolizing system caused by HCB are more persistent than those caused by TCB (I).
Effects on the reproductive capacity	The two chlorobiphenyls both have the ability to reduce the reproductive capacity in female mice, - the frequency of implanted ova is decreased in females given either HCB or TCB (III). Under the experimental conditions used in the experiments on mice, described in papers III and IV, effects on the reproductive capacity caused by HCB do not differ from those caused by TCB (III,IV). However, in pregnant rats given HCB the frequency of resorbed fetuses is higher than in pregnant rats given TCB (unpublished results, table 3).
Effects on growth	The two chlorobiphenyls both have the ability to promote the postnatal growth in young mice (V). HCB increases growth rate only in males, whereas TCB increases growth rate in both sexes. The birth weights of male mice born by mothers exposed to HCB are higher than those of male mice born by mothers exposed to TCB or by control mothers (V).

In addition to these findings, Brandt (1977) has shown that TCB, in contrast to HCB, is eliminated rapidly from the body. This indicates that TCB is transferred via the food chains to higher trophic levels to a much lesser extent than HCB.

Table 3. Frequency of resorbed fetuses in rats given daily doses of 2,4',5 - trichlorobiphenyl (TCB) or 2,2',4,4',5,5' - hexachlorobiphenyl (HCB), compared by means of chi-square test for heterogenity. The chlorobiphenyl administration started Day five of pregnancy (Day one = the day when a vaginal plug was found). The females were killed and dissected in late gestation (Day 19 - Day 20).

Chlorobiphenyl administered to the females	No. of females	No. of implantations	Resorbed fetuses No.	Resorbed fetuses per cent
None	11	146	5	3.4
TCB, 0.5 mg/day	10	134	5	3.7
HCB, 0.5 mg/day	10	129	22	17.1 [a]

[a] Significantly different from the corresponding values obtained from control females and from females given TCB ($p < 0.001$).

Apparently, HCB has in several respects more obvious effects than TCB and from a biological point of view the properties of TCB seem to be significantly less hazardous than those of HCB.

The results summarized in this thesis are obtained from laboratory experiments on mice and rats given structurally defined and highly purified chlorinated biphenyls. Thus, by means of these results it is not possible to give a correct and detailed prediction of the biological consequences connected with a possible restriction of the industrial use of PCBs, to such PCB preparations containing only biphenyls with a low chlorine content.

Monsanto Chemical Corporation, USA has manufactured a PCB preparation with a low chlorine content - MCS 1043. According to the manufacturer this product is a mixture of mono- and dichlorobiphenyls

but, by means of gas liquid chromatography and mass spectrometry it has been shown that MCS 1043 also contains trichlorobiphenyls (Reutergårdh personal communication).

Results from experiments, performed as described in paper I, have shown that under these conditions MCS 1043 has no detectable effect on the hepatic drug metabolizing system in the mouse (Örberg & Kihlström to be published). Experiments, designed to study effects of MCS 1043 on the reproductive function in mice have recently been completed and the preliminary results from these experiments suggest that small amounts of MCS 1043 have no effect on this function (Örberg & Kihlström to be published).

The animal body is able to metabolize chlorinated biphenyls, particularly those with a low chlorine content (Koeman et al. 1969, Hutzinger et al. 1972, de Freitas & Norstrom 1974). Thus, animals given e.g. MCS 1043 will be exposed not only to the parent substances but also to their metabolites.

Only limited information concerning biological effects and behaviour in biological systems of metabolites from chlorinated biphenyls is available. Brandt (1977, personal communication) has shown that a metabolite(s) of TCB is accumulated in the bronchial mucosa of mice and that another (?) metabolite(s) of the same compound is excreted into the uterine fluid of pregnant mice. Jensen and Jansson (1976) have presented results showing that certain metabolites of PCBs - methyl sulfones - are accumulated in the fat of seals and minks. The physiological significance of these findings is unknown.

To make it possible to form any definite idea about the biological hazards connected with the industrial use of PCB preparations with a low chlorine content it is necessary to study biological effects of an accumulation, if any, of metabolites from biphenyls with a low chlorine content.

The results from the experiments concerning effects of MCS 1043, indicate that the toxicity of this compound is very low. If the toxicity of metabolites from MCS 1043 would turn out to be very low as well, it is

most likely, if the use of PCBs will be restricted to PCB preparations with a low chlorine content, that MCS 1043 may fulfil the criteria for a "harmless" PCB preparation. This assumption is valid only if the disposal of PCBs will be kept at a low level.

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6. REFERENCES

Allen, J.R., and D.A. Barsotti: The effects of transplacental and mammary movement of PCBs on infant rhesus monkeys. Toxicology 1976, 6, 331-340.

Baird, J.D.: Some aspects of carbohydrate metabolism in pregnancy with special reference to the energy metabolism and hormonal status of the infant of the diabetic woman and the diabetogenic effect of pregnancy. J. Endocrinol. 1969, 44, 139-172.

Bennett, G.A., C.K. Drinker and M. Field Warren: Morphological changes in the livers of rats resulting from exposure to certain chlorinated hydrocarbons. J. Ind. Hyg. Toxicol. 1938, 20, 97-123.

Bickers, D.R., L.C. Harber A. Kappas and A.P. Alvares: Polychlorinated biphenyls: Comparative effects of high and low chlorine containing Aroclors on hepatic mixed function oxidase. Res. Commun. Chem. Path. Pharmacol. 1972, 3, 505-512.

Brandt, I.: Tissue localization of polychlorinated biphenyls, chemical structure related to pattern of distribution. Acta pharmacol. toxicol. 1977, 40, suppl. II 1-108.

Bruckner, J.V., K.L. Khanna and H.H. Cornish: Biological responses of the rat to polychlorinated biphenyls. Toxicol. appl. Pharmacol. 1973, 24, 434-448.

Chambon, Y.: Besoins endocriniens qualitatifs et quantitatifs de l'ovoimplantation chez la lapine. C.R. de la Soc. de biol. 1949, 143, 1172-1175.

Chen, T.S. and K.P. DuBois: Studies on the enzyme inducing effects of polychlorinated biphenyls. Toxicol. appl. Pharmacol. 1973, 26, 504-512.

Drinker, C.K., M. Field Warren and G. A. Bennett: The problem of possible systemic effects from certain chlorinated hydrocarbons. J. Ind. Hyg. Toxicol. 1937, 19, 283-299.

Ecobichon, D. J. and A. M. Comeau: Isomerically pure chlorobiphenyl congeners and hepatic function in the rat: Influence of position and degree of chlorination. Toxicol. appl. Pharmacol. 1975, 33, 94-105.

de Freitas, A.S. and R.J. Norstrom: Turnover and metabolism of polychlorinated biphenyls (PCBs) in relation to their chemical structure and the movement of lipids in the pigeon. Can. J. Physiol. Pharmacol. 1974, 52, 1080-1094.

Friend, M. and D.O. Trainer: Polychlorinated biphenyl: Interaction with duck hepatisis virus. Science 1970, 170, 1314-1316.

Gaunt, R.: Chemical control of growth in animals. In Dynamics of growth processes, 183-211. Ed. E.J. Boell Princeton, New Jersey, Princeton University Press 1954.

Grant, D.L. and W.E.J. Phillips: The effect of age and sex on the toxicity or Aroclor 1254, a polychlorinated biphenyl, in the rat. Bull. Environ. Contam. Toxicol. 1974, 12, 145-151.

Grant, D.L., W.E.J. Phillips and D.C. Villeneuve: Metabolism of a polychlorinated biphenyl (Aroclor 1254) mixture in the rat. Bull. Environ. Contam. Toxicol. 1971 b, 6, 102-112.

Grant, D.L., D.C. Villeneuve, K.A. McCully and W.E.J. Phillips: Placental transfer of polychlorinated biphenyls in the rabbit. Environ. Physiol. 1971 a, 1, 61-66.

Helle, E., M. Olsson and S. Jensen: DDT and PCB levels and reproduction in ringed seal from the Bothnian Bay. Ambio 1976, 5, 188-189.

Hubbard, H.L.: Chlorinated biphenyl and related compounds in Kirk-Othmer Encyclopedia of Chemical Technology, 2 nd edition 1964, 5, 289-297.

Hutzinger, O., D.M. Nash, S.Safe, A.S.W. de Freitas, R.J. Norstrom, D.J. Wildish and V. Zitko: Polychlorinated biphenyls: Metabolic behaviour of pure isomers in pigeons, rats and brook trout. Science 1972, 178, 312-313.

Inao, S.: Adrenocortical insufficiency induced in rats by prolonged feeding of Kanechlor (chlorobiphenyl). Kumamoto Med. J. 1970, 23, 27-31.

Jensen, S.: Report of a new chemical hazard. New Scientist 1966, 32, 612.

Jensen, S., J.E. Kihlström, M. Olsson, C. Lundberg and J. Örberg: Effects of PCB and DDT on mink (Mustela vison) during the reproductive season. Ambio 1977, 6, 239.

Jensen, S. and B. Jansson: Metyl sulfone metabolites of PCB and DDE. Ambio 1976, 5, 257-260.

Johnstone, G.J., D.J. Ecobichon and O. Hutzinger: The influence of pure polychlorinated biphenyl compounds on hepatic function in the rat. Toxicol. appl. Pharmacol. 1974, 28, 66-81.

Jonsson, Jr., H.T., J.E. Keil, R.G. Gaddy, C.B. Loadholt, G.R. Hennigar and E.M. Walker, Jr.: Prolonged ingestion of commercial DDT and PCB; Effects on progesterone levels and reproduction in the mature female rat. Arch. Environ. Contam. Toxicol. 1975/76 3, 479-490.

Kihlström, J.E., C. Lundberg, J. Örberg, P.O. Danielsson and J. Sydhoff: Sexual functions of mice neonatally exposed to DDT or PCB. Environ. Physiol. Biochem. 1975, 5, 54-57.

Kihlström, J.E., J. Örberg, C. Lundberg and P.O. Danielsson: Post-natal growth in mice sucking milk containing PCB or DDT. Ambio 1974, 3, 231-233.

Koeman, J.H., M.C. Ten Noe ver de Braun and R.H. de Vos: Chlorinated biphenyls in fish mussels and birds from the river Rhine and the Netherlands coastal area. Nature (Lond.) 1969, 221, 1126-1128.

Kuntzman, R., M. Jacobson, K. Schneidman and A.H. Conney: Similarities between oxidative drug-metabolizing enzymes and steroid hydroxylases in liver microsomes. J. Pharmacol. exp. Therap. 1964, 146, 280-285.

Lincer, J.L. and D.B. Peakall : Metabolic effects of polychlorinated biphenyls in the American kestrel. Nature (Lond.) 1970, 228, 783.

Linder, R.E., T.B. Gaines and R.D. Kimbrough: The effect of polychlorinated biphenyls on rat reproduction. Fd. Cosmet. Toxicol. 1974, 12, 63-77.

Liptrap, R.M. and J.I. Raeside : Increase in plasma testosterone concentration after injection of adrenocorticotrophin into the boar. J. Endocrinol. 1975, 66, 123-131.

Litterst, C.L., T.M. Farber, A.M. Baker and E.J. van Loon: Effect of polychlorinated biphenyls in hepatic microsomal enzymes in the rat. Toxicol. appl. Pharmacol. 1972, 23, 112-122.

McCormack, J.T. and G.S. Greenwald : Evidence for a preimplantation rise in oestradiol - 17β levels on day 4 of pregnancy in the mouse. J. Reprod. Fert. 1974, 41, 297-301.

Melvås, B. and I. Brandt: The distribution and metabolism of labelled polychlorinated biphenyls in mice and quails. In PCB conference II. National Swedish Environment Protection Board Publication 4 E, Stockholm, 1973, 87-100.

Nalbandov, A.V.: Endocrine control of implantation. In The biology of the blastocyst. 383-392. Ed. R.J. Blandau Chicago: University of Chicago Press 1971.

Noordhoek, J.: The effect of castration and testosterone on some components of the microsomal drug metabolizing enzymes in mice. Febs Letters 1972, 24, 225-259.

Nowicki, H.G. and A.W. Norman: Enhanced hepatic metabolism of testosterone 4-androstene-3, 17-dione and estradiol-17β in chickens pretreated with DDT or PCB. Steroids 1972, 19, 85-99.

Olsson, M., A.G. Johnels and R. Vaz: DDT and PCB levels in seals from Swedish waters. The occurrence of aborted seal pups. Proceedings from the symposium on the Seal in the Baltic. June 4-6, 1974, Lidingö, Sweden. National Swedish Environment Protection Board, SNV PM 591, 1975.

Örberg, J. and C. Lundberg: Some effects of DDT and PCB in the hormonal system in the male mouse. Environ. Physiol. Biochem. 1974, 4, 116-120.

Peakall, D.B.: PCB's and their environmental effects. CRC Crit. Rev. Environ. Contr. 1975, 5, 469-508.

Platonow, N.S., H.S. Funnell, D.H. Bullick, D.R. Arnott, P.W. Saschenbrecker and D.G. Grieve: Fate of polychlorinated biphenyls in dairy products precessed from the milk of exposed cows. J. Dairy Sci. 1971, 54, 1305-1308.

Platonow, N.S. and L.H. Karstad: Dietary effects of polychlorinated biphenyls on mink. Can. J. comp. Med. 1973, 37, 391-400.

Platonow, N.S., E.B. Meads, R.M. Liptrap and F. Lote: Effects of some commercial preparations of polychlorinated biphenyls in growing piglets. Can. J. comp. Med. 1976, 40, 421-428.

Ringer, R.K., R.J. Aulerich and M. Zabik: Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. Preprinte of papers presented at National Meeting Am. Chem. Soc. 1972, 12, 149.

Risebrough, R.W., P. Rieche, D.B. Peakall, S.G. Herman and M.N. Kirven: Polychlorinated biphenyls in the global ecosystem. Nature (Lond.) 1968, 220, 1098-1102.

Shaikh, A.A.: Estrone and estradiol levels in the ovarian venous blood from rats during estrous cycle and pregnancy. Biol. Reprod. 1971, 5, 297-307.

Smith, D.M. and J.D. Biggers: The oestrogen requirement for implantation and the effect of its dose on the implantation response in the mouse. J. Endocr. 1968, 41, 1-9.

Smyth, H.F.: The toxicity of certain benzene derivatives and related compounds. J. Ind. Hyg. 1931, 13, 87-96.

Street, J.C., F.M. Urry, D.J. Wagstaff and A.D. Blau: Comparative effects of polychlorinated biphenyls and organochlorine pesticides in induction of hepatic microsomal enzymes. Amer. Chem. Soc. 58th Annual Meeting Sept. 1969 Div. Pesticide Chem. 1969.

Vos, J.G. and Th. de Roij: Immunosuppressive activity of polychlorinated biphenyl preparation on the humoral immune response in guinea pigs. Toxicol. appl. Pharmacol. 1972, 21, 549-555.

Westöö, G., K. Norén and M. Andersson: Levels of organochlorine pesticides and polychlorinated biphenyls in margarine, vegetable oils, and some foods of animal origin on the Swedish market in 1967 - 1969. Vår föda 1970, 22, 9-31.

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A Comparison between some Effects of Two Pure Chlorobiphenyls (2,4′,5 - trichlorobiphenyl and 2,2′,4,4′,5,5′ - hexachlorobiphenyl) on the Hepatic Drug Metabolizing System in the Female Mouse

By

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Abstract: The effects of two pure chlorobiphenyls (2,4′,5-trichlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl) on the hepatic drug metabolizing system, and the elimination rate from the liver of the two chlorobiphenyls have been studied in the female mouse. The results obtained show that: 1. The two chlorobiphenyls are both potent inducers of the hepatic drug metabolizing system. 2. The hexachlorobiphenyl is a more potent inducer of the hepatic drug metabolizing system than the trichlorobiphenyl. 3. The trichlorobiphenyl is eliminated from the liver much faster than the hexachlorobiphenyl.

Key-words: Chlorobiphenyls – elimination rate – drug metabolizing system – mouse.

In most studies on the biological effects of polychlorinated biphenyls, technical preparations such as Aroclor, Phenoclor and Clophen have been used. These preparations are complex mixtures of about 80 different chlorobiphenyls, and they also contain some toxic impurities such as chlorinated dibenzofurans and chlorinated naphthalenes (Vos *et al.* 1970; Jensen & Sundström 1974). Some of the chlorobiphenyls have been synthesized and highly purified, and this has made it possible to study the biological effects of pure chlorinated biphenyls.

The purpose of the present investigation was to study differences, if any, between two pure chlorobiphenyls (2,4′,5-trichlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl) as to elimination rate from the liver, effects on a) the hepatic content of cytochrome P-450, b) the liver weight and c) the hexobarbital induced sleeping time in female mice.

Materials and Methods

Sexually mature female mice of the NMRI-strain were used. There were housed separately and fed pellets (standard food for rats and mice, Ewos Ltd.) and tap water *ad libitum*. In order to study the elimination rate from the liver of the two chlorobiphenyls, a total of 33 females were given orally 0.2 ml of a peanut oil solution containing 1.5 mg (5.8 μmol) 2,4′,5-trichlorobiphenyl and 1.5 mg (4.2 μmol) 2,2′,4,4′,5,5′-hexachlorobiphenyl. The animals were killed by a blow on the head at different intervals (22.5 min., 45 min., 1.5 hrs, 3.0 hrs, 6.0 hrs, 12.0 hrs, 24.0 hrs, 48.0 hrs, 96.0 hrs) after the administration of the drugs. The amounts of the two chlorobiphenyls in the liver were analysed by G. L. C. (Örberg & Kihlström 1973).

In order to study effects of the two chlorobiphenyls on the hepatic content of cytochrome P-450, on the hexobarbital induced sleeping time and on the liver weight, the animals were given orally, once a day for 4 consecutive days (day 1–4), 1.5 mg 2,4′,5-trichlorobiphenyl dissolved in 0.2 ml peanut oil (groups T_8 and T_{29}), 1.5 mg 2,2′,4,4′,5,5′-hexachlorobiphenyl dissolved in 0.2 ml peanut oil (groups H_8 and H_{29}) or 0.2 ml peanut oil (groups C_8 and C_{29}). The animals belonging to groups T_8, H_8 and C_8 were analysed 8 days after starting treatment and the animals belonging to groups T_{29}, H_{29} and C_{29} were analysed 29 days after starting treatment. The animals used when studying the hepatic content of cytochrome P-450 were killed by a blow on the head on the day of analysis. The liver was removed, weighed and homogenized with 25 ml cold (4°) 0.25 M sucrose solution in a glass-teflon homogenizer. The homogenate was centrifugated at 9,400 × g (4°) for 20 min. The supernatant was the centrifugated at 105,000 × g (4°) for 60 min. in an ultracentrifuge. After washing the pellet was suspended in 4 ml 0.25 M sucrose solution. The microsome suspension was then diluted (1:5) in KH_2PO_4 buffer (0.05 M, pH 7.5). The cytochrome P-450 content was measured according to Omura & Sato (1967). Proteins were determined by the procedure of Lowry *et al.* (1951), using bovine serum albumin as a protein standard.

When studying the hexobarbital induced sleeping time the animals were injected i. p. on the day of analysis with sodium hexobarbital (enhexymalum NFN) (evipan®), 100 mg/kg body weight. Immediately after the injection, the animals were placed on their backs in individual empty cages and the sleeping time was determined. On recovery, the animals were killed by a blow on the head and the liver was removed and weighed.

When studying the hepatic content of cytochrome P-450 and the hexobarbital induced sleeping time the animals were also used for studying the effect of the two chlorobiphenyls on the liver weight.

The results from the study on the elimination rate from the liver of the two chlorobiphenyls were analysed statistically by means of regression analysis. The cytochrome P-450 values, the liver weights and the hexobarbital induced sleeping times were analysed by means of the t-test.

Results and Discussion

The two chlorobiphenyls both reached their maximum concentrations in the liver 1.5 hrs after the drug administration. The concentration (mean ± S. D., p. p. m., liver fat weight basis) of the trichlorobiphenyl increased from 253.0 ± 20.5 p. p. m. – 0.38 hr post administration to 289.6 ± 46.4 p. p. m. –

1.5 hr post administration and the concentration of the hexachlorobiphenyl increaced from 43.0 ± 7.3 p. p. m. – 0.38 hr post administration to 867.0 ± 106.1 p. p. m. – 1.5 hrs post administration. The maximum concentration of the hexachlorobiphenyl was significantly higher than that of the trichlorobiphenyl ($P < 0.005$). Moreover, the elimination rate from the liver of the trichlorobiphenyl was much greater than that of the hexachlorobiphenyl (fig. 1).

These results support other investigations concerning the ability of the animal body to metabolize chlorinated biphenyls. Chlorobiphenyls with a low chlorine content, which appeared in Aroclor 1254, are more easily metabolized than chlorobiphenyls with a high chlorine content (GRANT *et al.* 1971; DE FREITAS & NORSTRÖM 1974). Results from experiments with pure chlorobiphenyls (4-mono-, 4,4′-di-, 2,2′,5,5′-tetra- and 2,2′,4,4′,5,5′-hexachlorobiphenyls) indicate that the formation of hydroxylated metabolites

Fig. 1. The disappearance of 2,4′,5-trichlorophenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl from the livers of female mice. The animals were given orally 0.2 ml of a peanut oil solution containing 1.5 mg 2,4′,5-trichlorobiphenyl and 1.5 mg 2,2′,4,4′,5,5′-hexachlorobiphenyl. They were killed at varying intervals after the drug administration and the amounts of the two chlorobiphenyls in the liver were analysed by GLC. The vertical bars represent the standard error of the mean. a) The regression coefficients differ significantly from each other ($P < 0.0005$).

Table 1.

Hepatic cytochrome P-450 content in female mice after oral administration for 4 consecutive days of 2,4′,5-trichlorobiphenyl (1.5 mg in 0.2 ml peanut oil/day, groups T_8 and T_{29}) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (1.5 mg in 0.2 ml peanut oil/day, groups H_8 and H_{29}) or peanut oil (0.2 ml/day, groups C_8 and C_{29}) compared by means of the t-test.

Group	No. of animals	Day of analysis*	Cyt. P-450 nmol/mg microsomal protein Mean ± S. D.
C_8	6	8	0.26 ± 0.05
T_8	6	8	0.40 ± 0.06[a]
H_8	6	8	0.49 ± 0.05[a,b]
C_{29}	5	29	0.26 ± 0.01
T_{29}	6	29	0.25 ± 0.05[d]
H_{29}	6	29	0.32 ± 0.05[c,e]

* The day when the oral administration of the drugs started was designated day 1.
a) Significantly different from C_8 ($P < 0.0025$)
b) Significantly different from T_8 ($P < 0.0125$)
c) Significantly different from C_{29}, T_{29} ($P < 0.025$)
d) Significantly different from T_8 ($P < 0.0005$)
e) Significantly different from H_8 ($P < 0.0005$)

in rats and in pigeons is fastest with monochlorobiphenyl, followed by the dichlorobiphenyl and the tetrachlorobiphenyl, and is slowest with the hexachlorobiphenyl (HUTZINGER *et al.* 1972).

Experimental animals, analysed on day 8, show significantly higher contents of hepatic cytochrome P-450 than the controls (table 1). The hexachlorobiphenyl treated animals also show significantly higher cytochrome P-450 values than those treated with trichlorobiphenyl. Similar differences between the effects of technical mixtures of polychlorinated biphenyls with low and high chlorine contents (Aroclor 1016 and Aroclor 1254) on the hepatic content of cytochrome P-450 have been reported by BRICKERS *et al.* (1972).

The cytochrome P-450 values from animals, analysed on day 29 (table 1), show that there is still a difference between the hexachlorobiphenyl treated animals and the controls, and also between the hexachlorobiphenyl treated animals and those treated with trichlorobiphenyl, whereas there is no difference between the trichlorobiphenyl treated animals and the controls. Table 1 also shows that a significant reduction in the hepatic cytochrome P-450 contents takes place between day 8 and day 29 in the experimental animals.

The effects of the drugs on the liver weight follow a very similar pattern (table 2). For example, the differences obtained on day 8 in liver weights

and in hepatic cytochrome P-450 contents between the controls and the trichlorobiphenyl treated animals have both disappeared on day 29, whereas the differences between the control and the hexachlorobiphenyl treated animals are still present on day 29.

On day 8 the hexobarbital induced sleeping times are significantly shorter in the experimental animals than in the controls, but there is no difference between the trichlorobiphenyl treated animals and the hexachlorobiphenyl treated animals (table 3). Hexachlorobiphenyl treated animals, studied on day 29, exhibit significantly shorter periods of sleep as compared with both the trichlorobiphenyl treated animals and the control animals, studied on day 29.

From the results presented in this study it is obvious that 2,2′,4,4′,5,5′-hexachlorobiphenyl is a more potent inducer of the hepatic drug metabolizing system than 2,4′,5-trichlorobiphenyl. Generally, the effects of the drug are primarily dependent on the number of moles, and not on the number of grams, of a substance present in a cell or an organ. From this point of view it is interesting to note that the animals from groups T_8 and T_{29} have received about 23.2 μmol 2.4′,5-trichlorobiphenyl, while the animals from groups H_8 and H_{29} have received only about 16.8 μmol 2,2′,4,4′,5,5′-hexachlorobiphenyl. This means, when comparing the effects of the two chloro-

Table 2.

Liver weights, expressed in terms of percentage of the body weight, from female mice after oral administration for 4 consecutive days of 2,4′,5-trichlorobiphenyl (1.5 mg in 0.2 ml peanut oil/day, groups T_8 and T_{29}) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (1.5 mg in 0.2 ml peanut oil/day, groups H_8 and H_{29}) or peanut oil (0.2 ml/day, groups C_8 and C_{29} compared by means of the t-test.

Group	No. of animals	Day of analysis*	Liver weight (percent of body weight)) Mean ± S. D.
C_8	14	8	4.50 ± 0.52
T_8	14	8	5.34 ± 0.55[a]
H_8	14	8	6.20 ± 1.02[a,b]
C_{29}	14	29	4.42 ± 0.36
T_{29}	14	29	4.54 ± 0.34[d]
H_{29}	14	29	5.09 ± 0.38[c,e]

* The day when the oral administration of the drugs started was designated day 1.

a) Significantly different from C_8 ($P < 0.0005$)
b) Significantly different from T_8 ($P < 0.01$)
c) Significantly different from C_{29}, T_{29} ($P < 0.0005$)
d) Significantly different from T_8 ($P < 0.0005$)
e) Significantly different from H_8 ($P < 0.0005$)

Table 3.

Sleeping times of female mice after oral administration for 4 consecutive days of 2,4′,5-trichlorobiphenyl (1.5 mg in 0.2 ml peanut oil/day, groups T_8 and T_{29}) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (1.5 mg in 0.2 ml peanut oil/day, groups H_8 and H_{29}) or peanut oil (0.2 ml/day, groups C_8 and C_{29}) and after injection (intraperitoneal) of hexobarbital (evipan®) 100 mg/kg body weight, compared by means of the t-test.

Group	No. of animals	Day of analysis*	Sleeping time minutes Mean ± S. D.
C_8	7	8	18.9 ± 6.3
T_8	8	8	7.8 ± 2.4[a]
H_8	6	8	9.0 ± 3.6[a]
C_{29}	6	29	18.5 ± 7.1
T_{29}	8	29	14.8 ± 6.4[c]
H_{29}	7	29	9.9 ± 1.9[b]

* The day when the oral administration of the drugs started was designated day 1.
a) Significantly different from C_8 ($P < 0.005$)
b) Significantly different from C_{29}, T_{29} ($P < 0.05$)
c) Significantly different from T_8 ($P < 0.01$)

biphenyls on the content of cytochrome P-450 in the liver (table 1, day 8), that the hexachlorobiphenyl molecule is about three times as effective as the trichlorobiphenyl molecule in increasing the hepatic cytochrome P-450 content. Moreover, the effects on the function studied remain for a longer time if caused by hexachlorobiphenyl than if caused by trichlorobiphenyl. It is most likely that the differences between effects of the two chlorobiphenyls on the hepatic drug metabolizing system are due to the fact that the elimination from the liver of the trichlorobiphenyl is much faster than that of the hexachlorobiphenyl (fig. 1).

On the basis of the results presented in this paper and on the results presented by JOHNSTONE *et al.* (1974), biphenyls with a low chlorine content might be less harmful to the environment than biphenyls with a high chlorine content. But before any conclusions can be drawn, more information is needed about the biological effects of biphenyls with a low chlorine content and biphenyls with a high chlorine content. Experiments designed to give data about: 1) transfer of 2,4′,5-trichlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl to foetuses and young via the placenta and the milk, respectively, and 2) the effects of the two drugs on the growth of young and on the reproductive capacity are in progress in this laboratory.

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REFERENCES

Brickers, D. R., L. C. Harber, A. Kappas & A. P. Alvares: Polychlorinated biphenyls: Comparative effects of high and low chlorine containing Aroclors on hepatic mixed function oxidase. *Res. Commun. Chem. Path. Pharmacol.* 1972, **3**, 505–512.

Carlson, R. W. & R. T. Duby: Embryotoxic effects of three PCB's in the chicken. *Bull. Environ. Toxicol. Contam.* 1973, **9**, 261–266.

de Freitas, A. S. & R. J. Norström: Turnover and metabolism of polychlorinated biphenyls (PCBs) in relation to their chemical structure and movement of lipids in the pigeon. *Can. J. Physiol. Pharmacol.* 1974, **52**, 1080–1094.

Grant, D. L., W. E. J. Phillips & D. C. Villeneuve: Metabolism of a polychlorinated biphenyl (Aroclor 1254) mixture in the rat. *Bull. Environ. Contam. Toxicol.* 1971, **6**, 102–112.

Hutzinger, O., D. M. Nash, S. Safe, A. S. de Freitas, R. J. Norström, D. J. Wildish & V. Zitko: Polychlorinated biphenyls: Metabolic behavior of pure isomers in pigeons, rats and brook trout. *Science* 1972, **178**, 312–313.

Jensen, S. & G. Sundström: Structures and levels of most chlorobiphenyls in two technical PCB products and in human adipose tissue. *Ambio* 1974, **3**, 70–76.

Johnstone, G. J., D. J. Ecobichon & O. Hutzinger: The influence of pure polychlorinated biphenyl compounds on hepatic function in the rat. *Toxicol. Appl. Pharmacol.* 1974, **28**, 66–81.

Keplinger, M. L., O. E. Fancher & J. C. Calandra: Toxicologic studies with polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.* 1971, **19**, 402–403.

Lowry, O. H., N. J. Rosebrough, A. L. Farr & R. J. Randall: Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.* 1951, **193**, 265–275.

Moron, M., G. Sundström & C. A. Wachtmeister: Polychlorinated biphenyls. VI. 2,3,7,8-Tetrachlorodibenzofuran, a critical byproduct in the synthesis of 2,2′,4,4′,5,5′-hexachlorobiphenyl by the Ullman reaction. *Acta Chem. Scand.* 1973, **27**, 3121–3122.

Omura, T. & R. Sato: The carbon monoxidebinding pigment of liver microsomes. *J. Biol. Chem.* 1967, **239**, 2370–2378.

Örberg, J. & J. E. Kihlström: Effects of long-term feeding of polychlorinated biphenyls (PCB, Clophen A 60) on the length of the oestrous cycle and on the frequency of implanted ova in the mouse. *Environ. Res.* 1973, **6**, 176–179.

Stalling, D. L. & F. L. Mayer: Toxicities of PCB to fish and environmental residues. *Environ. Health Perspectives* 1972, **1**, 159–164.

Sundström, G.: Polychlorinated biphenyls. II Synthesis of some tetra- and pentachlorobiphenyls. *Acta Chem. Scand.* 1973, **27**, 600–604.

Vos, J. G., J. H. Koeman, H. L. van der Maas, M. C. ten Noever de Brauw & R. H. de Vos: Identification and toxicological evaluation of chlorinated dibenzofuran and clorinated naphtalene in two commercial polychlorinated biphenyls. *Food Cosmet. Toxicol.* 1970, **8**, 625–633.

Acta pharmacol. et toxicol. 1977, **41**, 11–17.

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Effects of Pure Chlorobiphenyls (2,4′,5 - trichlorobiphenyl or 2,2′,4,4′,5,5′ - hexachlorobiphenyl) on the Disappearance of ^{14}C from the Blood Plasma after Intravenous Injection of 4-^{14}C-progesterone and on the Hepatic Drug Metabolizing System in the Female Rat

By

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Abstract: Effects of two pure chlorobiphenyls (2,4′,5-trichlorobiphenyl or 2,2′,4,4′,5,5′-hexachlorobiphenyl) on the hepatic drug metabolizing system and on the elimination rate of ^{14}C from the blood after intravenous injection of 4-^{14}C- progesterone have been studied in the female rat. The results obtained show that females pre-treated with small amounts of 2,2′,4,4′,5,5′-hexachlorobiphenyl (0.5 mg/day for 14 consecutive days) have significantly higher elimination rates of ^{14}C (originating from intravenous injection of 4-^{14}C-progesterone) from the blood plasma, significantly higher content of hepatic cytochrome P-450 and significantly heavier livers than control females. On the other hand, the results obtained from females pre-treated with small amounts of 2,4′,5-trichlorobiphenyl (0.5 mg/day for 14 consecutive days) do not differ significantly from the results obtained from control females.

Key-words: Chlorobiphenyls – progesterone half-life – drugmetabolizing system – rat.

KUNTZMAN *et al.* (1964) have shown that steroid hormones are normal substrates for microsomal enzymes in the hepatic drug metabolizing system. The activity in this system is stimulated by many different drugs, for instance by polychlorinated biphenyls (PCBs) (RISEBROUGH *et al.* 1968; LITTERST *et al.* 1972). Accordingly, the metabolism, *in vitro,* of sex steroids is increased in liver microsomes from birds pre-treated with PCBs (RISEBROUGH *et al.* 1968; LINCER & PEAKALL 1970; NOWICKI & NORMAN 1972). ÖRBERG & LUNDBERG (1974) have also reported results indicating an enhanced breakdown, *in vivo,* of testosterone in male mice exposed to PCBs.

The PCB preparations used in these experiments (Aroclor 1254, 1260, 1262 and Clophen A 60) are complex mixtures of many different chlorinated biphenyls (Sissons & Welti 1971; Jensen & Sundström 1974). Bickers *et al.* (1972) have shown in experiments on rats that a PCB preparation with a low chlorine content (Aroclor 1016, 16 % chlorine) is a less potent inducer of the hepatic drug metabolizing system than a PCB preparation with a moderate chlorine content (Aroclor 1254, 54 % chlorine). This means that effects of chlorinated biphenyls on steroid metabolism may be less pronounced if the chlorobiphenyl has a low chlorine content than if it has a moderate chlorine content.

The present work was designed to study the effects of two pure chlorobiphenyls (2,4′,5-trichlorobiphenyl or 2,2′,4,4′,5,5′-hexachlorobiphenyl) on the elimination rate of ^{14}C from the blood plasma after intravenous injection of 4-^{14}C-progesterone, on the hepatic content of cytochrome P-450 and on the liver weight in female rats.

Material and Methods

When studying the effects of the chlorobiphenyls on the liver weight and on the hepatic content of cytochrome P-450, 24 female Sprague Dawley rats, weighing 260–280 grams, were used. They were kept in individual cages and fed pellets (standard food for rats and mice, Ewos Ltd.) and tap water *ad libitum*. Once a day for 14 consecutive days the animals were given orally 0.5 mg 2,4′,5-trichlorobiphenyl dissolved in 0.1 ml peanut oil (8 females), 0.5 mg 2,2′,4,4′,5,5′-hexachlorobiphenyl dissolved in 0.1 ml peanut oil (8 females) or only 0.1 ml peanut oil (8 females). The equipment for the oral administration is described by Örberg & Kihlström (1973). The day after the last drug administration the females were killed by a blow on the head and the livers were removed and weighed. The microsomal fraction from 3–4 grams of liver was prepared as described earlier (Örberg 1976). The hepatic cytochrome P-450 content was measured according to Omura & Sato (1967). Proteins were determined by the procedure of Lowry *et al.* (1951), using bovine serum albumin as a protein standard. In order to determine the residual level of the two chlorobiphenyls, a piece of the liver (about 2 grams) was prepared and analysed (G. L. C.) as described by Örberg (unpublished results).

For studying the effect of the two chlorobiphenyls on the elimination rate of ^{14}C from the blood plasma after intravenous injection of 4-^{14}C-progesterone, 23 female Sprague Dawley rats, weighing 260–280 grams, were used. The feeding and the chlorobiphenyl treatment of these females were the same as described above. In order to decrease the stress factors associated with the usual methods of collecting blood from rats (heart puncture, tail vein puncture, tail- and toe-clippings), the blood was collected by means of a catheter permanently implanted in the left common carotid artery. The day before the last chlorobiphenyl administration the females were anaesthetized with ether and a small (1.5–2 cm) paramedian skin incision was made to the left of the midline on the ventral side of the neck. The left common carotid artery was located, gently separated from the vagus nerve and ligated just below the bifurcation into the external and internal carotid branches. The artery was elevated and streched by means of forceps, which was inserted under the vessel (from the left-hand side).

The forceps was then placed on the neck at right angles to the incision. The extended part of the artery was perforated (the pressure exerted by the shanks of the forceps on the wall of the vessel prevented blood leakage) by means of an injection needle (1 mm outer diameter) and about 30 mm of silicone tubing (20 cm - total length, 0.63 mm – inner diameter, 1.19 mm – outer diameter), filled with a heparin solution (25 IU heparin/ml 0.9 % NaCl solution) was introduced into the vessel. The catheter was tied into place with a ligature around the artery. Before closing the incision, the free end of the catheter was exteriorized through the skin between the scapulas. A plastic cylinder (27 mm – diameter, 15 mm – height) equipped with a screw cap was anchored to the skin (over the scapulas) by wire sutures. The catheter was flushed with heparin (25 IU heparin/ml 0.9 % NaCl solution), closed by means of a small clip and placed in the cylinder. Finally the cylinder was closed by means of the screw cap.

The day after the last chlorobiphenyl administration (two days after the surgical incision) the females were injected intravenously (under slight ether anaesthesia) in the tail with 0.3 μci (1.6 μg) 4-^{14}C-progesterone (The Radiochemical Center, Amersham, England) dissolved in 0.1 ml of a solution containing 0.9 % NaCl and 50 % ethanol. Serial blood samples (about 0.3 ml each) were collected in heparinized glass tubes by means of the catheter at different intervals (10 min., 20 min., 40 min., 60 min., 80 min., 120 min., 160 min.) after the injection of labelled progesterone. Immediately after a blood sample was drawn, the catheter was flushed with heparin (25 IU heparin/ml 0.9 % NaCl solution). The blood was centrifugated and 0.1 ml plasma was transferred to scintillating vials containing 10 ml liquid scintillator (Aquasol, New England Nuclear). After mixing, on a vortex mixer, the vials were placed in a liquid scintillation counter (Mark II, Nuclear Chicago) and the radioactivity in the samples was estimated. By re-estimating the radioactivity in the samples after the addition of a known activity (about 10,000 cpm, 4-^{14}C-progesterone) to each vial, the quench factors were determined. The disappearance curve for the radioactivity (semi log. plot) was plotted for each animal and the half-life in the plasma of radioactivity was calculated from the slope of the regression line.

All statistical analyses were performed by means of the t-test.

Results and Discussion

Females pretreated with 2,2′,4,4′,5,5′-hexachlorobiphenyl exhibit significantly greater elimination rates from the blood plasma of the radioactivity originating from 4-^{14}C-progesterone, significantly higher content of hepatic cytochrome P-450 and significantly heavier livers than control females (tables 1 and 2). On the other hand, the results obtained from females pre-treated with 2,4′5-trichlorobiphenyl do not differ significantly from the results obtained from control females (tables 1 and 2).

Since the hepatic cytochrome P-450 content (reflecting the activity in the hepatic drug metabolizing system) is increased in females exposed to 2,2′4,4′,5,5′-hexachlorobiphenyl (table 2), the short plasma ^{14}C half-life values obtained from these animals are certainly caused by an increased breakdown of 4-^{14}C-progesterone, followed by a rapid removal of progesterone metabolites from the blood. In addition to the hepatic drug metabolizing system,

Table 1.

The plasma ^{14}C half-life values, the regression coefficients and the corresponding correlation coefficients from curves (semi log. plot) originating from the disappearance of ^{14}C from the plasma in female rats, injected intravenously with 0.3 μci 4-^{14}C-progesterone. The females were pretreated with 2,4′,5-trichlorobiphenyl (TCB) or 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB). The plasma ^{14}C half-life values were compared by means of the t-test.

Females Treatment	No.	Plasma ^{14}C half-life min. Mean ± S. D.	Regression coefficient ($\times 10^{-3}$) Mean ± S. D.	Correlation coefficient Mean ± S. D.
None	8	55.1 ± 11.6	−5.71 ± 1.33	−0.982 ± 0.017
Exposed to TCB	6	49.6 ± 8.9	−6.23 ± 1.12	−0.982 ± 0.010
Exposed to HCB	9	45.7 ± 10.0 [a]	−6.81 ± 1.23	−0.981 ± 0.021

[a] Significantly different from controls ($P < 0.05$).

Table 2.

Hepatic cytochrome P-450 content and liver weight in female rats after oral administration for 14 consecutive days of 2,4′,5-trichlorobiphenyl (TCB) (0.5 mg in 0.1 ml peanut oil/day), 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB) (0.5 mg in 0.1 ml peanut oil/day) or only peanut oil (0.1 ml/day). The results were compared by means of the t-test.

Females Treatment	No.	Cytochrome P-450 nmol/mg microsomal protein Mean ± S. D.	Liver weight percent of body weight Mean ± S. D.
None	8	0.45 ± 0.09	3.65 ± 0.28
Exposed to TCB	8	0.45 ± 0.04	3.71 ± 0.13
Exposed to HCB	8	0.68 ± 0.08 [a]	4.12 ± 0.32 [b]

[a] Significantly different from the corresponding value obtained from the controls/ the trichlorobiphenyl treated females ($P < 0.0005$).

[b] Significantly different from the corresponding value obtained from the controls/ the trichlorobiphenyl treated females ($P < 0.0025$).

the actual amount of endogenous progesterone in the blood may also affect the elimination rate of exogenous progesterone. However, the analysis (radioimmunoassay, RIA) of blood samples, drawn immediately before the injection of labelled progesterone, showed that there was no significant difference in the concentration of plasma progesterone between females exposed to 2,2′,4,4′,5,5′-hexachlorobiphenyl and control females ($P > 0.70$, plasma progesterone, ng/ml plasma, mean ± S. D.: controls – 22.0 ± 12.8, females exposed to 2,2′,4,4′,5,5′-hexachlorobiphenyl – 24.3 ± 6.0). Thus, it is not very probable that differences in blood progesterone levels have contributed to the difference in ^{14}C elimination rates obtained between the females from these groups. (The concentration of plasma progesterone in control females did not differ significantly from the concentration of plasma progesterone in females exposed to 2,4′,5-trichlorobiphenyl ($P > 0.30$) – plasma progesterone, ng/ml plasma, mean ± S. D.: 29.7 ± 14.6).

It is interesting to note that under the experimental conditions used here, the 2,4′,5-trichlorobiphenyl has no apparent effect on the hepatic drug metabolizing system in the female rat. In female mice the 2,4′,5-trichlorobiphenyl is eliminated much faster from the liver than the 2,2′4,4′,5,5′-hexachlorobiphenyl (ÖRBERG 1976). As the residual level of chlorobiphenyl in the livers from females exposed to 2,4′,5-trichlorobiphenyl is much lower than in livers from females exposed to 2,2′,4,4′,5,5′-hexachlorobiphenyl (table 3), there is good reason to believe that the 2,4′,5-trichlorobiphenyl is also eliminated much faster from the liver than the 2,2′,4,4′,5,5′-hexachloro-

Table 3.

Concentrations of 2,4′,5-trichlorobiphenyl (TCB) or 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB) in the livers of female rats, given orally TCB (0.5 mg in 0.1 ml peanut oil/day) or HCB (0.5 mg in 0.1 ml peanut oil/day) for 14 consecutive days. The results were compared by means of the t-test.

Females Treatment	No.	% extrac. fat Mean (Range)	p. p. m. TCB or HCB Mean ± S. D. in extrac. fat	in fresh tissue
Exposed to TCB	6	3.7 (3.4–4.3)	3.4 ± 1.1	0.13 ± 0.04
Exposed to HCB	6	3.9 (2.9–4.3)	196 ± 32 [a]	3.9 ± 0.54 [a]

[a] Significantly different from the corresponding value obtained from animals exposed to TCB ($P < 0.0005$).

biphenyl in the rat. A rapid elimination of 2,4′,5-trichlorobiphenyl from the liver is probably the reason for the absence of detectable effects of the trichlorobiphenyl on the hepatic drug metabolizing system.

The results of this investigation indicate on the one hand, that effects of small amounts of 2,4′,5-trichlorobiphenyl on the breakdown of progesterone are without physiological significance in the female rat, and on the other, that small amounts of 2,2′,4,4′,5,5′-hexachlorobiphenyl, via induction of the hepatic drug metabolizing system, may interfere with progesterone dependent functions, such as the maintenance of the pregnancy in the female rat. But this does not mean that biological effects of 2,4′,5-trichlorobiphenyl are always less hazardous than the biological effects of 2,2′,4,4′,5,5′-hexachlorobiphenyl. Results from experiments on mice, foetally and neonatally exposed to 2,4′,5-trichlorobiphenyl or 2,2′,4,4′,5,5′-hexachlorobiphenyl indicate that the reproductive capacity is decreased only in animals exposed to 2,4′,5-trichlorobiphenyl (ÖRBERG, unpublished results).

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REFERENCES

Bickers, D. R., L. C. Harber, A. Kappas & A. P. Alvares: Polychlorinated biphenyls: Comparative effects of high and low chlorine containing Aroclors on hepatic mixed function oxidase. *Res. Commun. Chem. Path. Pharm.* 1972, **3**, 505–512.

Jensen, S. & G. Sundström: Structures and levels of most chlorobiphenyls in two technical PCB products and in human adipose tissue. *Ambio* 1974, **3**, 70–76.

Kuntzman, R., M. Jacobson, K. Schneideman & A. H. Conney: Similarities between oxidative drug-metabolizing enzymes and steroid hydroxylases in liver microsomes. *J. Pharmacol. exp. Therap.* 1964, **146**, 280–285.

Lincer, J. L. & D. B. Peakall: Metabolic effects of polychlorinated biphenyls in the american kestrel. *Nature* (Lond.) 1970, **228**, 783.

Litterst, C. L., T. M. Farber, A. M. Baker & E. J. van Loon: Effect of polychlorinated biphenyls on hepatic microsomal enzymes in the rat. *Toxicol. Appl. Pharmacol.* 1972, **23**, 112–122.

Lowry, O. H., N. J. Rosebrough, A. L. Farr & R. J. Randall: Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.* 1951, **193**, 265–275.

Nowicki, H. G. & A. W. Norman: Enhanced hepatic metabolism of testosterone,

4-androstene-3, 17-dione, and estradiol-17β in chickens pretreated with DDT or PCB. *Steroids* 1972, **19**, 85–99.

Omura, T. & R. Sato: The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.* 1967, **239**, 2370–2378.

Örberg, J. & J. E. Kihlström: Effects of long-term feeding polychlorinated biphenyls (PCB, Clophen A 60) on the length of the oestrus cycle and on the frequency of implanted ova in the mouse. *Environ. Res.* 1973, **6**, 176–179.

Örberg, J. & C. Lundberg: Some effects of DDT and PCB on the hormonal system in the male mouse. *Environ. Physiol. Biochem.* 1974, **4**, 116–120.

Örberg, J.: A comparison between some effects of two pure chlorobiphenyls (2,4′,5-trichlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl) on the hepatic drug metabolizing system in the female mouse. *Acta pharmacol. et toxicol.* 1976, **39**, 145–151.

Risebrough, R. W., P. Rieche, D. B. Peakall, S. G. Herman & M. M. Kirvin: Polychlorinated biphenyls in the global ecosystem. *Nature (Lond.)* 1968, **220**, 1098–1102.

Sissons, D. & D. Welti: Structural identification of polychlorinated biphenyls in commercial mixtures by gas-liquid chromatography, nuclear magnetic resonance and mass spectrometry. *J. Chromatogr.* 1971, **60**, 15–32.

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Effects of pure chlorobiphenyls (2,4′,5 - trichlorobiphenyl and 2,2′,4,4′,5,5′- hexachlorobiphenyl) on the reproductive capacity in female mice.

By
Jan Örberg

ABSTRACT

Effects of two pure chlorobiphenyls (2,4′,5 - trichlorobiphenyl and 2,2′,4,4′,5,5′- hexachlorobiphenyl) on the reproductive capacity in female mice have been studied. The results obtained show that females given 0.5 mg 2,4′,5 - trichlorobiphenyl (TCB) / day or 0.5 mg 2,2′,4,4′,5,5′- hexachlorobiphenyl (HCB) / day for six consecutive days, starting on the first day of pregnancy, have significantly lower frequencies of implanted ova than the control females, whereas the frequency of implanted ova does not differ either between females given 0.05 mg/day of TCB or HCB and the control females, or between females given TCB 0.05 mg/day or

0.5 mg/day and those given the corresponding dose of HCB. Females given 0.5 mg/day of TCB or HCB, respectively, also have significantly higher contents in the livers of cytochrome P-450, on Day four of pregnancy, than the control females. The results also show that, under the experimental conditions used, neither the frequency of pregnancies nor the number of implanted ova per pregnant female differ significantly between the different groups.

Key-words: PCB - chlorobiphenyls - reproductive capacity - drug metabolizing system - mouse.

INTRODUCTION

Technical preparations of polychlorinated biphenyls (PCB) and also pure chlorobiphenyls are potent inducers of the hepatic drug metabolizing system (Risebrough et al. 1968, Litterst et al. 1972, Johnstone et al. 1974, Örberg 1976). As the steroid hormones are normal substrates for the drug metabolizing enzymes (Kuntzman et al. 1964), not only the breakdown of foreign compounds but also the breakdown of endogenous substances such as progesterone, estradiol and testosterone is increased in animals pretreated with chlorinated biphenyls (Risebrough et al. 1968, Örberg & Lundberg 1974, Örberg & Ingvast 1977). This means that the hormonal milieu essential for the implantation of blastocysts in the wall of the uterus (Smith 1968, Smith & Biggers 1968, Hedlund & Nilsson 1971, McCormack & Greenwald 1974) may be altered in females exposed to inducers of the drug metabolizing enzymes. Such an alteration might result in a decreased frequency of implanted ova (Smith 1968, Smith & Biggers 1968).

Biphenyls with a low chlorine content are less potent inducers of the drug metabolizing enzymes than biphenyls with a moderate chlorine content (Bickers et al. 1972, Örberg 1976). This indicates that effects of chlorinated biphenyls on physiological functions controlled

by steroid hormones and caused by biphenyls with a low chlorine content might be less pronounced than those caused by biphenyls with a moderate chlorine content.

The present work was designed to compare effects of two pure chlorobiphenyls (2,4',5 - trichlorobiphenyl and 2,2',4,4',5,5'- hexachlorobiphenyl) on the reproductive capacity in female mice.

MATERIAL AND METHODS

In this experiment about 230 female mice of the NMRI-strain were used. They were placed five to a cage with a male of proven fertility. The females were checked each morning for the presence of a vaginal plug. The day on which one was found was designated Day one of pregnancy. After mating, the females were housed separately and divided into five groups - one control group and four experimental groups (TCB 0.05, TCB 0.50, HCB 0.05, and HCB 0.50). Once a day from Day one of pregnancy the animals were given per os 0.1 ml peanut oil (controls), 0.05 mg or 0.50 mg 2,4',5 - trichlorobiphenyl (TCB) dissolved in 0.1 ml peanut oil (TCB 0.05 and TCB 0.50, respectively) and 0.05 mg 0.50 mg 2,2',4,4',5,5'- hexachlorobiphenyl (HCB) dissolved in 0.1 ml peanut oil (HCB 0.05, and HCB 0.50, respectively).

In order to study the activity in the hepatic drug metabolizing system at the time of the preimplantation rise in plasma 17-β-estradiol level (McCormack & Greenwald 1974), 11 - 12 females from each group were killed by a blow on the head on Day four of pregnancy (the day after their last chlorobiphenyl administration). The liver was removed, weighed and the microsomal fraction of the liver was prepared as described earlier (Örberg 1976). The cytochrome P-450 content was measured accordingly to Omura & Sato (1967). Proteins were determined by the procedure of Lowry et al. (1952) using bovine serum albumin as a protein standard.

The remaining females were killed on Day seven of pregnancy (the day after their last chlorobiphenyl administration). The liver was removed, weighed and frozen pending residue analysis and the number of corpora lutea and placentae were counted, considering each placenta as indicating an implantation. The corpora lutea were counted by means of a magnifying glass (magnification: four times). As the corpus luteum regresses if no implantation takes place, it is not possible with this method to determine the number of ova shed at the time of mating in females without implantations. This means that the results concerning the frequency of implanted ova are obtained only from females with at least one implantation site.

The residue levels of the two chlorobiphenyls in the livers from the females were determined as described earlier (Örberg 1977).

The statistical analyses were performed by means of chi-square test for heterogenity or t-test. When applying t-test to small samples (degrees of freedom less than 15) the t-value was corrected for small samples according to de V. Weir (1960).

RESULTS

Females given 0.5 mg 2,4′,5 - trichlorobiphenyl (TCB)/day exhibited a significantly lower frequency of implanted ova than the control females (Table 1) and females given 0.5 mg 2,2′,4,4′,5,5′- hexachlorobiphenyl (HCB)/day exhibited a significantly lower frequency of implanted ova than both the control females and the females given 0.05 mg HCB/day (Table 1). However, there were no differences in the frequency of implanted ova either between the females given 0.05 mg TCB/day and those given 0.05 mg HCB/day, or between the females given 0.5 mg TCB/day and those given 0.5 mg HCB/day (Table 1). Table 1 also shows that there were no differences between the different groups in either the number of implanted ova/pregnant female, or the frequency of pregnancies.

As seen in Table 2, the contents of hepatic cytochrome P-450 were significantly higher in the females from groups TCB 0.50, HCB 0.05 and HCB 0.50 than in the females from the control group. Females given the high dose (0.50 mg/day, groups TCB 0.50 and HCB 0.50) of one of the two chlorobiphenyls showed significantly higher cytochrome P-450 values than those given the low dose (0.05 mg/day, groups TCB 0.05 and HCB 0.05) of the same chlorobiphenyl, whereas the cytochrome P-450 values obtained from females given TCB did not differ significantly from those obtained from females given the same dose of HCB (Table 2). Table 2 also shows that the females from group HCB 0.50 had significantly greater liver weight/body weight ratios than the females from the control group, from group TCB 0.50 and from group HCB 0.05.

The results from the residue analyses are shown in Table 3. As seen from this table, the females given HCB had significantly higher residue levels than those given the same dose of TCB.

DISCUSSION

The idea that a decreased reproductive capacity in female mice, exposed to chlorinated hydrocarbons such as PCB and DDT, is caused by a disturbance of the balance of the sex steroids is supported by the results from this study. - Females from group TCB 0.50 and group HCB 0.50 exhibited low frequencies of implanted ova (Table 1) and high activity in the hepatic drug metabolizing system at the time of the preimplantation rise in plasma 17-β - estradiol level (Table 2).

The low frequency of implanted ova among females from group TCB 0.50 may not only be caused by the trichlorobiphenyl. - As seen in Table 1, the females from this group had somewhat greater number of corpora lutea per pregnant female than the controls. Although the number of corpora lutea did not differ

significantly between the two groups ($p < 0.20$, t-test, two-tailed), it cannot be excluded that the great number of ovulated eggs in females from group TCB 0.50 has contributed to the low frequency of implanted ova. (The number of ovulated eggs was not affected by the chlorobiphenyls as the chlorobiphenyl administration started after the ovulation.)

As seen from Table 1, the two chlorobiphenyls had detectable effects only on the frequency of implanted ova and not on either the frequency of pregnancies or the number of implanted ova/pregnant female. This means that when recording only the frequency of pregnancies and the number of implanted ova/pregnant female or the litter sizes, an actual decline in the reproductive capacity in the females may not be discovered.

The fact that there were no significant differences in the hepatic content of cytochrome P-450 either between females from group TCB 0.05 and those from group HCB 0.05, or between the females from group TCB 0.50 and those from group HCB 0.50 is in conflict with results obtained from an earlier investigation showing that HCB is a more potent inducer of the hepatic drug metabolizing system than TCB (Örberg 1976). A possible explanation for these contradictory results may be found in differences in experimental conditions between the present work and the work of Örberg (1976). - In the present investigation mated female mice, divided into four groups were given (per os) 0.05 mg/day or 0.50 mg/day of TCB or HCB, respectively, for three consecutive days, starting on the first day of pregnancy. The hepatic contents of cytochrome P-450 were then determined the day after the last chlorobiphenyl administration (Day four of pregnancy). In the investigation of Örberg (1976) virgin female mice, divided into two groups, were given (per os) 1.5 mg/day of TCB or HCB, respectively, for four consecutive days. Then the hepatic contents of cytochrome P-450 were not determined until four days after the last chlorobiphenyl administration. Thus in these two experiments the hormonal status of the females, which has been shown to affect the activity in the drug metabolizing system (Noordhoek 1972), probably differs. Moreover,

the amounts of the chlorobiphenyls given to the females and the time elapsed from the last chlorobiphenyl administration to the killing of the females differ between the experiments.

The great difference in residue levels in the livers between females given 2,4′,5 - trichlorobiphenyl and those given 2,2′,4,4′,5,5′- hexachlorobiphenyl (Table 3) are certainly caused by differences in elimination rate from the liver between the two chlorobiphenyls. - The trichlorobiphenyl is eliminated much faster than the hexachlorobiphenyl (Örberg 1976).

Under the experimental conditions used in this study both the trichlorobiphenyl (with a low chlorine content) and the hexachlorobiphenyl (with a moderate chlorine content) decrease the frequency of implanted ova in female mice. However, no significant difference between the effects of the two chlorobiphenyls could be demonstrated. This fact does not support the hypothesis that effects on reproductive functions caused by biphenyls with a moderate chlorine content are more pronounced than those caused by biphenyls with a low chlorine content.

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Table 1.

Frequencies of pregnancies and implantation of ova in mice given 2,4',5 - trichlorobiphenyl (TCB) or 2,2',4,4,5,5'-hexachlorobiphenyl (HCB) for six consecutive days starting on the day when a vaginal plug was found (Day one of pregnancy). The females were killed and dissected on Day seven of pregnancy.

Females, treatment	No. of mated females	Pregnant females: Pregnancies No.	Pregnancies % [a]	Corpora lutea Total no.	Corpora lutea Mean ± S.D. [b]	Implanted ova Total no.	Implanted ova Mean ± S.D. [b]	Implanted ova % [a]
Controls, 0.1 ml peanut oil/day	56	40	71.4	453	11.33 ± 2.07	418	10.45 ± 2.21	92.3
TCB 0.05, 0.05 mg TCB in 0.1 ml peanut oil/day	20	15	75.0	164	10.93 ± 2.09	152	10.13 ± 2.80	92.7
TCB 0.50, 0.50 mg TCB in 0.1 ml peanut oil/day	40	28	70.0	337	12.04 ± 1.86	296	10.57 ± 2.87	87.8 [c]
HCB 0.05, 0.05 mg HCB in 0.1 ml peanut oil/day	20	14	70.0	163	11.64 ± 1.78	154	11.00 ± 1.36	94.5
HCB 0.50, 0.50 mg HCB in 0.1 ml peanut oil/day	32	24	75.0	284	11.83 ± 2.14	238	9.92 ± 2.73	83.8 [d]

a/ Analysed statistically by means of chi-square test for heterogenity.

b/ Analysed statistically by means of t-test.

c/ Significantly different from controls ($p < 0.001$).

d/ Significantly different from controls ($p < 0.001$) and from HCB 0.05 ($p < 0.005$).

Table 2.

Hepatic cytochrome P-450 content and liver weight (Day four) in mated female mice after oral administration of 2,4′,5 - trichlorobiphenyl (TCB) or 2,2′,4,4′,5,5′- hexachlorobiphenyl (HCB) for three consecutive days starting on the day when a vaginal plug was found (Day one). The results were compared by means of t-test.

Females, treatment	No. of livers analysed	Cytochrome P-450 nmol/mg microsomal protein Mean ± S.D.	No. of livers weighed	Liver weight percent of body weight Mean ± S.D.
Controls, 0.1 ml peanut oil/day	11	0.48 ± 0.11	12	4.86 ± 0.32
TCB 0.05, 0.05 mg TCB in 0.1 ml peanut oil/day	11	0.53 ± 0.11	10	4.84 ± 0.50
TCB 0.50, 0.50 mg TCB in 0.1 ml peanut oil/day	12	0.92 ± 0.20 a/	10	4.99 ± 0.38
HCB 0.05, 0.05 mg HCB in 0.1 ml peanut oil/day	11	0.61 ± 0.12 b/	12	4.93 ± 0.62
HCB 0.50, 0.50 mg HCB in 0.1 ml peanut oil/day	12	0.97 ± 0.23 c/	10	5.37 ± 0.46 b, d/

a/ Significantly different from the corresponding values obtained from the controls and TCB 0.05 ($p < 0.0005$).

b/ Significantly different from the corresponding value obtained from the controls ($p < 0.005$).

c/ Significantly different from the corresponding values obtained from the controls and HCB 0.05 ($p < 0.0005$).

d/ Significantly different from the corresponding values obtained from TCB 0.50 and HCB 0.05 ($p < 0.05$).

Table 3.

Concentrations of 2,4′,5 - trichlorobiphenyl (TCB) or 2,2′,4,4′,5,5′ - hexachlorobiphenyl (HCB) in livers from pregnant females given TCB or HCB for six consecutive days starting Day one of pregnancy, compared by means of t-test. The t-values are corrected for small samples according to de V. Weir (1960).
(n = number of livers analysed.)

Females, treatment	n	% extrac. fat Mean (Range)	ppm TCB or HCB Mean ± S.D. in extract. fat	in fresh tissue
TCB 0.05, 0.05 mg TCB in 0.1 ml peanut oil/day	7	4.4 (3.6 - 5.3)	0.82 ± 0.66 a/	0.035 ± 0.027 a/
TCB 0.50, 0.50 mg TCB in 0.1 ml peanut oil/day	8	4.9 (4.0 - 5.8)	4.2 ± 1.2 b/	0.20 ± 0.04 b/
HCB 0.05, 0.05 mg HCB in 0.1 ml peanut oil/day	6	3.5 (2.5 - 4.2)	117 ± 38 b/	3.9 ± 0.88 b/
HCB 0.50, 0.50 mg HCB in 0.1 ml peanut oil/day	5	5.4 (4.2 - 6.7)	707 ± 97	39 ± 11

a/ Significantly different from the corresponding values obtained from TCB 0.50 and HCB 0.05 ($p < 0.0005$).

b/ Significantly different from the corresponding value obtained from HCB 0.50 ($p < 0.0005$).

REFERENCES

Bickers, D.R., L.C. Harber, A. Kappas & A.P. Alvares: Polychlorinated biphenyls: Comparative effects of high and low chlorine containing Aroclors on hepatic mixed function oxidase. Res. Commun. Path. Pharm. 1972, 3, 505-512.

Hedlund, K. & O. Nilsson: Hormonal requirements for the uterine attachment reaction and blastocyst implantation in the mouse, hamster and guinea-pig. J. Reprod. Fert. 1971, 26, 267-269.

Johnstone, G.J., D.J. Ecobichon & O. Hutzinger: The influence of pure chlorinated biphenyl compounds on hepatic function in the rat. Toxicol. appl. Pharmacol. 1974, 28, 66-81.

Kuntzman, R., M. Jacobson, K. Schneidman & A.H. Conney: Similarities between oxidative drug metabolizing enzymes and steroid hydroxylases in liver microsomes. J. Pharmacol. exp. Ther. 1964, 146, 280-285.

Litterst, C.L., T.M. Farber, A.M. Baker & E.J. van Loon: Effect of polychlorinated biphenyls on hepatic microsomal enzymes in the rat. Toxicol. appl. Pharmacol. 1972, 23, 112-122.

Lowry, O.H., N.J. Rosebrough, A.L. Farr & R.J. Randall: Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 1951, 193, 265-275.

McCormack, J.T. & G.S. Greenwald : Evidence for a preimplantation rise in oestradiol-17β levels day 4 of pregnancy in the mouse. J. Reprod. Fert. 1974, 41, 297-301.

Noordhoek, J.: The effect of castration and testosterone on some components of the microsomal drug metabolizing enzyme system in mice. Febs Letters 1972, 24, 255-259.

Omura, T. & R. Sato : The carbon monoxide binding pigment of liver microsomes. J. Biol. Chem. 1967, 239, 2370-2378.

Örberg, J.: A comparison between some effects of two pure chlorobiphenyls (2,4',5 - trichlorobiphenyl and 2,2',4,4',5,5'- hexachlorobiphenyl) on the hepatic drug metabolizing system in the female mouse. Acta pharmacol. toxicol. 1976, 39, 145-151.

Örberg, J.: Placental and mammary transfer of two PCBs (2,4',5 - TCB and 2,2',4,4',5,5'- HCB) and their effect on the reproductive capacity in mice. Ambio 1977, 5, 278-280.

Örberg, J. & C. Ingvast: Effects of pure chlorobiphenyls (2,4',5 - trichlorobiphenyl or 2,2',4,4',5,5'- hexachlorobiphenyl) on the disappearance of ^{14}C from the blood plasma after intravenous injection of 4-^{14}C-progesterone and on the hepatic drug metabolizing system in the female rat. Acta pharmacol. toxicol. 1977, 40, in press.

Örberg, J. & C. Lundberg: Some effects of DDT and PCB on the hormonal system in the male mouse. Environ. Physiol. Biochem. 1974, 4, 116-120.

Risebrough, R.W., P. Rieche, D.B. Peakall, S.G. Herman & M.N. Kirven: Polychlorinated biphenyls in the global ecosystem. Nature (Lond.) 1968, 220, 1098-1102.

Smith, D.M.: The effect of the time of oestrogen injection on implantation in ovariectomized pregnant mice. J. Endocr. 1968, 41, 11-15.

Smith, D.M. & J.D. Biggers : The oestrogen requirement for implantation and the effects of its dose on the implantation response in the mouse. J. Endocr. 1968, 41, 1-9.

de V. Weir, J.B.: Standardized t. Nature (Lond.) 1960, 185, 558.

Placental and Mammary Transfer of Two PCBs (2,4′, 5-TCB and 2,2′, 4,4′, 5,5′-HCB) and Their Effect on Reproductive Capacity in Mice

Report

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Polychlorinated biphenyls (PCB) are known to be transferred from the mother to the fetuses and sucklings via the placenta and the milk. This means that mothers given PCB expose their young to these substances before birth and also during the period of lactation. In experiments on pregnant rabbits it has been shown that the commercial PCB preparation Aroclor 1221, which contains 21 percent chlorine, is accumulated in fetal tissues to a much lesser extent than Aroclor 1254, which contains 54 percent chlorine. This may indicate that chlorinated biphenyls with a low chlorine content are less harmful to animals than those with a moderate chlorine content.

In the present work the placental and mammary transfer of two pure chlorobiphenyls—2,4′,5-trichlorobiphenyl (TCB) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB)—were studied in mice. In addition, the frequency of pregnancies and the number of fetuses per female among mice prenatally and postnatally exposed to either of these two substances were recorded.

From the results obtained it is clear that fetuses and sucklings of females given the HCB are exposed to much greater amounts of chlorobiphenyls than those of females given the TCB. The results also indicate that the enzymes essential for the breakdown of the TCB are already present in sucklings less than 12 days after birth. The chlorobiphenyls had no demonstrated effect on the reproductive capacity. However, this does not mean that chlorobiphenyls given to pregnant females or to nursing mothers are harmless to the offspring.

Polychlorinated biphenyls are known to cross the placenta in different species, *eg* rabbit (1), mouse (2, 3), rhesus monkey (4) and cow (5). It has also been shown that milk from women (6), rhesus monkeys (4) and cows (7) contain polychlorinated biphenyls. It is most likely that these substances also occur in milk from other species.

As polychlorinated biphenyls are widely distributed in the global ecosystem (8, 9), most mammals are exposed to these substances as fetuses and sucklings. Only limited information concerning the effects of prenatal and postnatal PCB exposure is available. Villeneuve *et al* (10), working with rabbit fetuses, could not demonstrate any differences in hepatic enzyme activity between fetuses exposed to PCB and unexposed controls. Decreased reproductive capacity and increased growth rate in mice postnatally exposed to PCB have been reported by Kihlström *et al* (11, 12).

In experiments on pregnant rabbits given a commercial PCB preparation—either Aroclor 1221 (containing 21 percent chlorine) or Aroclor 1254 (containing 54 percent chlorine)—Grant *et al* (1) found that Aroclor 1254 was accumulated in fetal tissue to a much greater extent than Aroclor 1221. From that result, one would expect the effects of prenatal PCB exposure to be less pronounced if the PCB preparation has a low chlorine content. In experiments on adult rats Bickers *et al* (13) showed that a PCB mixture with a 16 percent chlorine content (Aroclor 1016) is a less potent inducer of the hepatic drug metabolizing system than one with a 54 percent chlorine content (Aroclor 1254).

The present investigation studies the transfer in mice of two pure chlorobiphenyls, 2,4′,5-trichlorobiphenyl (TCB) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB), via the placenta and the milk, to fetuses and sucklings. The frequency of pregnancies and the number of fetuses per female among mice prenatally postnatally exposed to either of the two pure chlorobiphenyls were also recorded.

EXPERIMENTAL

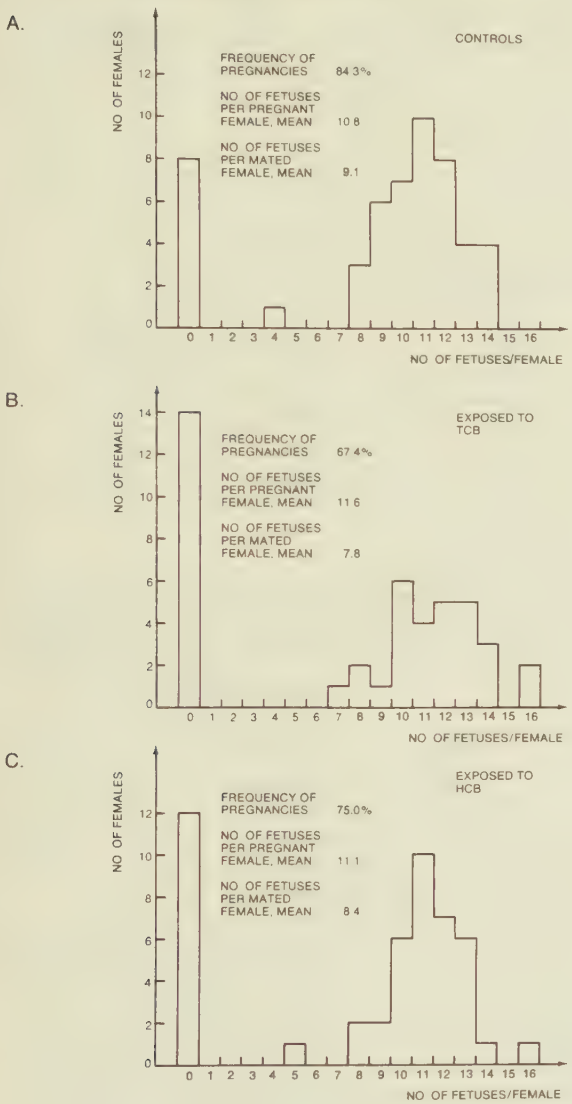
Mice of the NMRI strain were used. They were kept in individual cages, unless otherwise stated, and given pellets (standard food for rats and mice, Ewos Ltd) and tap water freely.

To study the placental transfer of 2,4′,5-trichlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl, 21 pregnant mice were given orally, once a day, from day five to day 19 of pregnancy, either 0.05 mg of TCB (10 females) or 0.05 mg of HCB (11 females). (The day when a vaginal plug was found was designated day one of pregnancy.) The equipment for the oral administration is described by Örberg and Kihlström (14). Four hours after the last chlorobiphenyl administration the females were killed and the liver and two fetuses (located closest to the cervix in each horn of the uterus) were removed, weighed and frozen pending residue analysis. In order to form a sample large enough for analysis, the two fetuses made up one unit.

To study the transfer of the chlorobiphenyls to the sucklings via the milk, 25 mothers and their litters were used. Owing to difficulties in obtaining sufficient amounts of milk from the mammary gland for analysis, the milk in the stomachs of the young was used instead. On the day of birth the number of young per litter was adjusted to ten. The mothers were given orally, once a day for 12 days, 0.05 mg of TCB (14 mothers) or 0.05 mg of HCB (11 mothers), starting on the day they gave birth. Four hours after the last chlorobiphenyl administration the mothers and two young per litter were killed. The livers of the mothers and the livers and stomach contents of the young were collected, weighed and frozen pending residue analysis. In the analysis, the livers of the two litter mates were used as one unit, and the stomach contents as another.

The livers and the fetuses were cut into pieces and homogenized with 6 ml of acetone in a Potter-Elvehjem glass homogenizer. The homogenizer was then rinsed with 5 + 5 ml of acetone. The homogenate and the rinsing acetone were transferred to a glass tube and concentrated to about 8 ml under a stream of nitrogen. After the addition of 4 ml of hexane and 5 ml of water, the tube was closed by means of a screw cap with a teflon disk and then rotated about its short axis at 14 rpm for 30 minutes. The tube was then centrifuged at 500 g for five minutes and the hexane transferred to an empty graduated glass tube. The sample was then extracted with an additional 6 ml of hexane (mixed on a vortex mixer 10 seconds, rotated 10 minutes) and centrifuged at 500 g for five minutes. The

Figure 1A–C. Number of fetuses per female in the control group and the groups exposed to TCB and HCB. The female and the male used for the mating were prenatally and postnatally exposed to the same chlorobiphenyl—either 2,4',5-trichlorobiphenyl (TCB) or 2,2',4,4',5,5'-hexachlorobiphenyl (HCB). Frequency of pregnancies compared by means of chi-square test for heterogenity, number of fetuses per pregnant/mated female compared by means of U-test.


A.
CONTROLS
FREQUENCY OF PREGNANCIES 84.3%
NO. OF FETUSES PER PREGNANT FEMALE, MEAN 10.8
NO. OF FETUSES PER MATED FEMALE, MEAN 9.1
NO. OF FEMALES
NO. OF FETUSES/FEMALE
B.
EXPOSED TO TCB
FREQUENCY OF PREGNANCIES 67.4%
NO. OF FETUSES PER PREGNANT FEMALE, MEAN 11.6
NO. OF FETUSES PER MATED FEMALE, MEAN 7.8
NO. OF FEMALES
NO. OF FETUSES/FEMALE
C.
EXPOSED TO HCB
FREQUENCY OF PREGNANCIES 75.0%
NO. OF FETUSES PER PREGNANT FEMALE, MEAN 11.1
NO. OF FETUSES PER MATED FEMALE, MEAN 8.4
NO. OF FEMALES
NO. OF FETUSES/FEMALE

resulting hexane solution was added to the first. The combined hexane solutions were then concentrated to 3 ml in a waterbath (60°C) under a stream of nitrogen. 1.0 ml of this solution was transferred to a pre-weighed cap of aluminum. When all the hexane had evaporated, the cap was weighed again and the fat content estimated. 2 ml of fuming sulfuric acid (10 percent sulfur trioxide) was added to the remaining 2 ml of hexane solution, and the tube was then closed by means of a screw cap with a teflon disk. The contents of the tube were gently mixed on a vortex mixer. After centrifugation (800 g, 5 minutes), the hexane was decanted and ready for injection into a gas chromatograph.

The stomach contents from the young were placed in a glass tube containing 2 ml of hexane and 1 ml of formic acid: acetonitril (1:3). After mixing (vortex mixer, 1 minute) the glass tube was rotated about its short axis (14 rpm) for 30 minutes. From this point, the sample preparation followed the schedule described above.

The gas chromatograph used was a Varian 2700 with an electron capture detector. The column was packed with a mixture of SF 96:QF1 (1:3) as the stationary phase on Chromosorb W AW (80/100 mesh). The temperatures of the injector block, column and the ^{3}H-detector were 195°C, 150°C, and 210°C respectively, and nitrogen was used as the carrier gas.

The experiment, designed to study effects of the two chlorobiphenyls on the frequency of pregnancies and on the number of fetuses per female, started with 35 pregnant mice. Once a day, from day five of pregnancy to day 22 post partum (the day of birth was designated day one post partum), the females, divided into three groups, were given orally 0.05 mg of TCB, 0.05 mg of HCB or the corresponding volume (0.1 ml) of the peanut oil vehicle. On the day of birth the number of young per litter was adjusted to ten. At weaning, day 22, the male and female young were separated from each other, and the period of chlorobiphenyl exposure ended. After reaching sexual maturity, the animals were placed in pairs (both the female and the male had been prenatally and postnatally exposed to the same chlorobiphenyl), in separate cages. The male was removed on the day a vaginal plug was found and each male was allowed to mate only one female. The females were killed and dissected on day ten or 11 of pregnancy and the number of fetuses was counted.

The statistical analyses were performed by means of the t-text, U-test (15, 16) or chi-square test for heterogenity.

RESULTS

The chlorobiphenyl residue levels in livers and fetuses of females given the HCB were much higher than those of females given the TCB (Table 1). Table 2 shows that sucklings of mothers given HCB had significantly higher residue levels of chlorobiphenyls in the livers and in the contents of the stomachs than sucklings of mothers given TCB. As seen, also in Table 2, the livers of the sucklings exhibited higher residue levels of chlorobiphenyls than the livers of the mothers and the difference in hepatic chlorobiphenyl residue levels between the mother and her own sucklings was significant if the mothers had been given HCB, but not if the mothers had been given TCB (Table 3). There were no significant differences in the number of fetuses per pregnant female, the number of fetuses per mated female or the frequency of pregnancies in females from the three different groups (Figure 1).

DISCUSSION

From these results it is obvious that fetuses and sucklings of females given low doses of HCB are exposed to greater amounts of chlorobiphenyls than those of females given similar doses of TCB. This does not necessarily mean that the HCB molecule has physical and chemical properties more favorable to placental and mammary transfer than the TCB molecule. As chlorobiphenyls with a low

chlorine content are more easily metabolized and eliminated than those with a moderate chlorine content (17, 18, 19), the average residue levels of chlorobiphenyls are likely to be much higher in females exposed to HCB than in females exposed to TCB (see Table 1, residue levels in the livers). This indicates that the chlorobiphenyl residue levels in fetuses and milk merely reflect the residue levels of the mother animals.

The results presented in Tables 2 and 3 indicate that the breakdown/elimination of TCB is much easier than that of HCB, also in young mice. This idea is also supported by the fact that the ratio between the residue levels in the livers of sucklings from the two groups (HCB/TCB; 90/6.4 = 14.1) is twice as great as the ratio between the residue levels in the contents of the stomachs of sucklings from the two groups (HCB/TCB; 33/5.2 = 6.3) (Table 2). The fact that the difference in the hepatic chlorobiphenyl residue level between sucklings and their own mother is small if the mother had been given TCB (Table 3), may imply that the ability of the young to metabolize TCB does not differ significantly from that of the adults. Although the drug metabolizing system in mice is not completely developed until two to three months after birth (20), enzymes essential for the breakdown of TCB are apparently already present in sucklings less than 12 days after birth.

Under the experimental conditions in this study there were no demonstrated effects of either of the two chlorobiphenyls on the reproductive capacity (Figure 1). However, this does not mean that chlorobiphenyls given to pregnant females or to nursing mothers are harmless to the offspring. Kihlström *et al* (12) have shown decreased frequencies of implanted ova in mice nursed by mothers given a commercial PCB preparation (Clophen A 60) and in experiments on minks it has been shown that postnatal mortality is higher among sucklings of bitches given feed containing polychlorinated biphenyls than among sucklings of control bitches (21).

On the basis of the results from this study it is most likely that the biological hazards of the industrial use of chlorobiphenyls would decrease if the use were restricted to chlorobiphenyls with a low chlorine content.

Table 1. Concentrations of 2, 4′,5-trichlorobiphenyl (TCB) or 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB) in pregnant female mice after oral administration of TCB or HCB (0.05 mg in 0.1 ml of peanut oil/day) for 15 consecutive days, starting day 5 of pregnancy, compared by means of the t-test. (n = number of specimens analyzed.)

	LIVER				FETUS			
		% extractable fat	ppm TCB or HCB Mean ± S D			% extractable fat	ppm TCB or HCB Mean ± S D	
	n	Mean (range)	in extractable fat	in fresh tissue	n	Mean (range)	in extractable fat	in fresh tissue
Exposed to TCB	10	4.8 (3.5–5.9)	7.0±5.4	0.33±0.25	10	1.4 (1.2–1.6)	3.0±1.2	0.04±0.02
Exposed to HCB	11	4.3 (3.5–5.1)	166±29*	7.2±1.3*	10	1.5 (1.2–1.8)	49±9.4*	0.72±0.16*

* Significantly different from the corresponding value obtained from the TCB treated females ($p < 0.0005$)

Table 2. Concentrations of 2,4′,5-trichlorobiphenyl (TCB) or 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB) in mothers and in their sucklings after oral administration of TCB or HCB (0.05 mg in 0.1 ml of peanut oil/day) to the mothers, for 12 consecutive days, starting on the day of delivery, compared by means of the t-test. (n = number of specimens analyzed.)

	LIVER (MOTHER)				LIVER (YOUNG)				CONTENT OF THE STOMACH (YOUNG)			
		% extractable fat	ppm TCB or HCB Mean S ± D			% extractable fat	ppm TCB or HCB Mean ± S D			% extractable fat	ppm TCB or HCB Mean ± S D	
	n	Mean (range)	in extractable fat	in fresh tissue	n	Mean (range)	in extractable fat	in fresh tissue	n	Mean (range)	in extractable fat	wet weight
Exposed to TCB	12	3.8 (3.3–4.3)	4.1±2.3	0.15±0.08	14	4.2 (3.4–5.4)	6.4±4.1	0.27±0.20	12	27 (13–37)	5.2±4.1	1.6±1.4
Exposed to HCB	11	3.8 (3.2–4.4)	56±33*	2.1±1.3*	11	4.1 (3.2–5.5)	90±39*	3.7±1.8*	11	33 (23–43)	33±18*	10±5.4*

* Significantly different from the corresponding value obtained from animals exposed to TCB ($p < 0.0005$)

Table 3. Difference* in hepatic chlorobiphenyl residue level between sucklings and their mothers, compared by means of the t-test. The mothers were given orally either 0.05 mg 2,4′,5-trichlorobiphenyl (TCB)/day or 0.05 mg 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB)/day for 12 consecutive days, starting on the day of delivery. The mother and the young were killed four hours after the last administration of the chlorobiphenyl.**

Chlorobiphenyl administered to the mothers	No. of samples	Difference in hepatic chlorobiphenyl residue level between sucklings and their mothers Mean ± S D ppm in extractable fat	ppm in fresh tissue
TCB	12	2.3±5.8	0.12±0.26
HCB	9	31 ±38***	1.6±2.1***

* The differences are calculated by subtracting the residue level in the liver of the mother from that of her own sucklings.
** The t-value is corrected for small samples according to de V Weir (22).
*** The difference is statistically significant ($p < 0.05$).

References and Notes:

1. D L Grant, D C Villeneuve, K A McCully, W E J Phillips, *Environmental Physiology and Biochemistry* **1**, 61 (1971).
2. B Melvås, J Brandt, *PCB Conference II* (National Swedish Environment Protection Board, Stockholm, 1973) Publication 4 E, pp 87–100.
3. J Brandt, *Acta Pharmacologica et Toxicologica* **40**, Supplement II (1977).
4. J R Allen, D A Barsotti, *Toxicology* **6**, 331 (1976).
5. N S Platonow, N Y Chen, *Veterinary Record* **92**, 69 (1973).
6. G Westöö, K Norén, M Andersson, *Vår föda* **2–3**, 9 (1970) (in Swedish, summary in English).
7. N S Platonow, H S Funnel, D H Bullock, D R Arnott, P W Saschenbrecker, D G Grieve, *Journal of Dairy Science* **54**, 1305 (1971).
8. R W Risebrough, P Reiche, D B Peakall, S G Herman, M N Kirven, *Nature (London)* **220**, 1098 (1968).
9. D B Peakall, *C R C Critical Reviews in Environmental Control* **5**, 469 (1975).
10. D C Villeneuve, D L Grant, W E J Phillips, M L Clark, D J Clegg, *Bulletin of Environmental Contamination and Toxicology* **2**, 120 (1971).
11. J E Kihlström, J Örberg, C Lundberg, P O Danielsson, *Ambio* **3**, 231 (1974).
12. J E Kihlström, C Lundberg, J Örberg, P O Danielsson, J Sydhoff, *Environmental Physiology and Biochemistry* **5**, 54 (1975).
13. D R Bickers, L C Harber, A Kappas, A P Alvares, *Research Communications in Chemical Pathology and Pharmacology* **3**, 505 (1972).
14. J Örberg, J E Kihlström, *Environmental Research* **6**, 176 (1973).
15. H B Mann, D R Whitney, *Annals of Mathematics and Statistics* **18**, 50 (1947).
16. S Siegel, *Nonparametric Statistics for the Behavioral Sciences* (Mc Graw-Hill Book Company, New York, 1956) p 116.
17. D L Grant, W E J Phillips, D C Villeneuve, *Bulletin of Environmental Contamination and Toxicology* **6**, 102 (1971).
18. O D Hutzinger, D M Nash, S Safe, A S de Freitas, R J Norström, D J Wildish, V Zitko, *Science* **178**, 312 (1972).
19. J Örberg, *Acta Pharmacologica et Toxicologica* **38**, 145 (1976).
20. W R Jondorf, R P Maickel, B B Brodie, *Biochemical Pharmacology* **1**, 352 (1958).
21. S Jensen, J E Kihlström, C Lundberg, M Olsson, J Örberg, *Ambio* **6**, 239 (1977).
22. J B de Weis, *Nature* **185**, 558 (1960).

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Effects of pure chlorobiphenyls (2,4´5 - trichlorobiphenyl and 2,2´4 4´5,5´-hexachlorobiphenyl) on the post-natal growth in mice.

By
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ABSTRACT

The post-natal growth rate of young mice, pre- and post-natally exposed to either 2,4´5 - trichlorobiphenyl or 2,2´4 4´5,5´- hexachlorobiphenyl has been studied. The results show that both the chlorobiphenyls have the ability to increase the post-natal growth rate in mice and they indicate that this ability of 2,4´5 - trichlorobiphenyl is most pronounced in females whereas that of 2,2´4,4´5,5´-hexachlorobiphenyl is most pronounced in males.

Key-words: PCB - chlorobiphenyls - growth rate - mouse.

INTRODUCTION

There are many results from different laboratories showing that polychlorinated biphenyls (PCB) affect the growth in different animal species. In pigs, mice and salmon polychlorinated biphenyls act as growth promoting substances whereas in minks and monkeys they decrease the growth rate (Ringer et al. 1972, Kihlström et al. 1974, Allen & Barsotti 1976, Gruger, Jr. et al. 1976, Platonow et al. 1976). The physiological reasons for these diverse effects of PCB on growth are not known.

Commercial PCB preparations used in most of these experiments are complex mixtures of many different chlorinated biphenyls, which differ from one another with regard both to the number and to the positions of the chlorine substituents (Sissons & Welti 1971, Jensen & Sundström 1974). Moreover some commercial PCB preparations also contain chlorinated dibenzofurans and chlorinated naphtalenes (Vox et al. 1970). This means that it is not possible to point out the substance or substances, in the PCB mixture, responsible for the effects on growth.

Results from some experiments on rats and mice indicate that biological effects caused by chlorinated biphenyls with a low chlorine content are less pronounced than those caused by biphenyls with a moderate chlorine content (Bickers et al. 1972, Johnstone et al. 1974, Örberg 1976, Örberg & Ingvast 1977). With this in mind I have designed an experiment to compare effects of two pure chlorobiphenyls (2,4′5 - trichlorobiphenyl and 2,2′4,4′5,5′- hexachlorobiphenyl) on the post-natal growth in mice.

MATERIAL AND METHODS

The experiment started with 39 pregnant mice of the NMRI-strain. They were kept in individual cages and given pellets (Standard food for rats and mice, Ewos Ltd) and water ad libitum. Every day, from Day five of pregnancy (the day when a vaginal plug was found was designated Day one of pregnancy) to

Day 22 post partum (the day of birth was designated Day one post partum), females, divided into three groups, were given per os 0.05 mg 2,4',5 - trichlorobiphenyl (TCB), 0.05 mg 2,2',4,4',5,5'- hexachlorobiphenyl (HCB) or the corresponding volume (0.1 ml) of the peanut oil vehicle, respectively. The equipment for the oral administration is described by Örberg and Kihlström (1973). Less than 18 hours after parturition the number of young per litter was adjusted to ten. The young were weighed individually (± 0.01 g - young not older than 25 days, ± 0.1 g - young older than 25 days) three or four times a week, starting on the day of parturition (Day one) and ending 34 days later. On Day five the young were marked individually by cutting holes in their ears. At weaning, Day 22, the males and females were placed in different cages, each one containing ten individuals and the period of chlorobiphenyl exposure ended.

As the growth curve during the studied period can be regarded as being composed of two different straight lines - one from Day five to Day 20 and the other from Day 21 to Day 35, - the results from these two periods have been analysed separately (Table 1). By means of regression analysis the intercepts, the regression coefficients and the correlation coefficients of the growth curves were determined. From these data the body weights of all young on Day five, on Day 20, on Day 21 and on Day 35 were calculated. The average growth rates of each young during the two periods were computed in two different ways: 1. Absolute growth rate - expressed as the gain in body weight (in grams) per day and equal to the slope of the growth curve (g/day), 2. Relative growth rate - defined as the gain in body weight (in grams) during the period expressed as per cent of the body weight in the middle of the period, divided by the length of the period (%/day). The relative growth rate was determined by means of the calculated weights on Day five, on Day 20, on Day 21 and on Day 35.

RESULTS

The average growth rates are presented in Table 1. During the second period, from Day 21 to Day 35, the relative growth rates of male young of mothers

given either HCB or TCB (henceforth called HCB males and TCB males, respectively) were significantly higher than that of male young of control mothers (henceforth called control males). During the same period the absolute growth rate of HCB males was also significantly higher than that of control males. Female young of mothers given TCB (henceforth called TCB females) exhibited significantly higher growth rates (absolute and relative growth rate) during this period than both female young of control mothers and those of mothers given HCB (henceforth called control females and HCB females, respectively), but the growth rates of HCB females did not differ from those of control females. During the first period, from Day five to Day 20, there were no significant differences in growth rates either between the young females or between the young males from the three different groups.

The actual body weights of the young at different ages are given in Table 2. At birth (Day one) HCB males were significantly heavier than both control males and TCB males. The same difference in body weights between TCB males and HCB males also existed on Day 21 and on Day 35. On Day 35 HCB males were also significantly heavier than control males. At birth there were no significant differences in body weights between the young females from the three groups, but on Day 21 HCB females exhibited significantly higher body weights than both control females and TCB females. On Day 35 HCB females and also TCB females were significantly heavier than control females.

DISCUSSION

From the results of this study it is not possible to form any definite idea about the physiological reasons for the stimulating effects on the growth rate of the two chlorobiphenyls. However, the fact that HCB females during the second period had growth rates equal to those of control females and significantly lower than those of TCB females, makes it possible to eliminate some explanations to the results.

It is well known that glucocorticoids e. g. cortisol and corticosterone stimulate the catabolism of proteins. This is probably one of the main reasons for their growth retardant effects (Gaunt 1954). This means that decreased plasma

levels of glucocorticoids in animals may result in increased growth. Such a reduction of the plasma levels of glucocorticoids could be caused by an increased breakdown or/and a decreased synthesis of these hormones.

The steroid hormones are normal substrates for the hepatic drug metabolizing enzymes (Kuntzman et al. 1964). As the activity of these enzymes is increased in animals exposed to chlorinated biphenyls (Street et al. 1969, Johnstone et al. 1974), it can not be excluded that the plasma levels of glucocorticoids may be subnormal in such animals. Sanders and Kirkpatrick (1975) have also showed decreased plasma levels of corticoids in mice exposed to a commercial PCB preparation - Aroclor 1254. However, as HCB is a more potent inducer of the hepatic drug metabolizing system than TCB (Örberg 1976), it is very unlikely that the effects of TCB on growth rate are due to reduced plasma levels of glucocorticoids as a result of a TCB-induced enhancement of the breakdown of glucocorticoids.

Inao (1970) has shown that the synthesis of corticosterone is reduced in rats given a commercial PCB preparation - Kaneclor 400. This reduction is probably due to a direct effect of PCB on the function of the adrenal cortex. This idea is supported by the fact that many pure ^{14}C-labelled chlorobiphenyls (2,2',4,4'- tetrachlorobiphenyl, 2,2',3,4,4',6 - and 2,3',4,4',5',6 - hexachlorobiphenyl and 2,2',3,3',4,4',5,6'- octachlorobiphenyl) are heavily accumulated in the adrenal cortex of mice (Melvås & Brandt 1973, Brandt 1977). However, in this case the difference in growth rate between TCB females and HCB females can probably not be explained by decreased plasma levels of glucocorticoids owing to a decreased hormone synthesis as a result of a direct effect of TCB on the adrenal cortex as only HCB and not TCB is accumulated in the adrenal cortex in mice (Brandt 1977).

It is interesting to note that not only the post-natal growth rate but apparently also the pre-natal growth rate of mice are affected by chlorobiphenyls. - HCB males were significantly heavier at birth than both control males and TCB males (Table 2). Fetuses of females given HCB under the same experimental conditions as in this study contained on Day 19 of pregnancy about 15 times more chlorobiphenyls than did fetuses of mothers given TCB. On the same day of pregnancy the residue level in the liver of pregnant females given HCB was

also much higher than that of females given TCB (Örberg 1977). These facts may, at least, partly contribute to the explanation of the difference in birth weight between HCB males and TCB males.

The physiological mechanisms responsible for the high birth weight of males born by mothers given HCB are probably different from those responsible for the enhanced growth rate of HCB males observed during the period from Day 21 to Day 35.

In order to obtain more information about effects of chlorobiphenyls on prenatal growth, experiments concerning the body composition of newborn mice of mothers exposed to chlorobiphenyls are in progress in this laboratory.

The results from the present study indicate on the one hand that there are no differences between TCB and HCB with regard to their ability to increase the growth rate in young mice and on the other that this ability of TCB is most pronounced in females whereas that of HCB is most pronounced in males.

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Table 1.

Growth rates of young mice born and nursed by mothers given either 2,4′,5 - trichlorobiphenyl (TCB) or 2,2′,4,4′,5,5′ - hexachlorobiphenyl (HCB), compared by means of t-test (two tailed).

Chlorobiphenyl administered to the mothers	Young no.	Young sex	Period: Day five - Day 20 Absolute growth rate [a] (g/day) Mean ± S.D.	Period: Day five - Day 20 Relative growth rate (%/day) Mean ± S.D.	Period: Day 21 - Day 35 Absolute growth rate [a] (g/day) Mean ± S.D.	Period: Day 21 - Day 35 Relative growth rate (%/day) Mean ± S.D.
None	53	male	0.423 ± 0.039 (0.9756 - 0.9974)	6.508 ± 0.471	0.954 ± 0.119 (0.9748 - 0.9992)	5.464 ± 0.418
HCB, 0.05 mg/day	54	male	0.428 ± 0.043 (0.9703 - 0.9984)	6.519 ± 0.391	1.005 ± 0.109 [b] (0.9760 - 0.9993)	5.676 ± 0.395 [c]
TCB, 0.05 mg/day	52	male	0.418 ± 0.038 (0.9567 - 0.9975)	6.597 ± 0.611	0.979 ± 0.114 (0.9815 - 0.9996)	5.697 ± 0.405 [c]
None	52	female	0.409 ± 0.047 (0.9722 - 0.9966)	6.454 ± 0.549	0.686 ± 0.078 (0.9833 - 0.9992)	4.545 ± 0.381
HCB, 0.05 mg/day	47	female	0.422 ± 0.038 (0.9752 - 0.9985)	6.522 ± 0.383	0.715 ± 0.096 (0.9710 - 0.9989)	4.593 ± 0.518
TCB, 0.05 mg/day	33	female	0.414 ± 0.034 (0.9487 - 0.9967)	6.642 ± 0.484	0.760 ± 0.098 [c,d] (0.9821 - 0.9995)	4.861 ± 0.468 [c,d]

a/ Regression coefficient of growth curve. The corresponding correlation coefficients are given between brackets (range).

b/ Significantly different from the corresponding value obtained from males/females of control mothers ($p < 0.025$).

c/ Significantly different from the corresponding value obtained from males/females of control mothers ($p < 0.01$).

d/ Significantly different from the corresponding value obtained from females of HCB-treated mothers ($p < 0.05$).

Table 2.

Body weights of young mice born and nursed by mothers given either 2,4′,5 - trichlorobiphenyl (TCB) or 2,2′,4,4′,5,5′- hexachlorobiphenyl (HCB), compared by means of t-test (two tailed). (n = number of young weighed this particular day, Day one = day of birth.)

Chlorobiphenyl administered to the mothers	Young sex	Body weight Mean ± S.D. n	Day one	n	Day 21	n	Day 35
None	male	71	1.48 ± 0.12	40	10.76 ± 0.73	40	23.6 ± 1.9
HCB, 0.05 mg/day	male	60	1.53 ± 0.13 [a,b]	44	10.94 ± 0.77 [c]	44	24.5 ± 1.5 [a,b]
TCB, 0.05 mg/day	male	66	1.48 ± 0.17	43	10.45 ± 0.95	43	23.6 ± 2.2
None	female	68	1.45 ± 0.13	36	10.25 ± 0.82	41	19.5 ± 1.5
HCB, 0.05 mg/day	female	53	1.46 ± 0.14	39	10.70 ± 0.86 [a,b]	39	20.6 ± 1.4 [a]
TCB, 0.05 mg/day	female	33	1.45 ± 0.16	24	10.14 ± 1.08	24	20.7 ± 1.8 [a]

a/ Significantly different from the corresponding value obtained from males/females of control mothers ($p < 0.025$).

b/ Significantly different from the corresponding value obtained from males/females of TCB treated mothers ($p < 0.05$).

c/ Significantly different from the corresponding value obtained from males of TCB treated mothers ($p < 0.025$).

REFERENCES

Allen, J.R. & D.A. Barsotti: The effects of transplacental and mammary movement of PCBs on infant rhesus monkeys. Toxicology 1976, 6, 331-340.

Bickers, D.R., L.C. Harber, A. Kappas & A.P. Alvares: Polychlorinated biphenyls: Comparative effects of high and low chlorine containing Aroclors on hepatic mixed function oxidase. Res. Commun. Chem. Path. Pharmacol. 1972, 3, 505-512.

Brandt, I.: Tissue localization of polychlorinated biphenyls. Chemical structure related to pattern of distribution. Acta pharmacol. toxicol. 1977, 40, supplement II.

Gaunt, R.: Chemical control of growth in animals, in Dynamics of growth processes 183-211. Ed. Boell, E.J. Princeton, New Jersey, Princeton University Press, 1954.

Gruger, Jr. E.H., T. Hruby, & M. L. Karrick: Sublethal effects of structurally related tetrachloro-, pentachloro-, and hexachloro-biphenyl on juvenil coho salmon. Environ. Sci. Techn. 1976, 10, 1033-1037.

Inao, S.: Adrenocortical insufficiency induced in rats by prolonged feeding of Kanechlor (chlorobiphenyl). Kumamoto Med. J. 1970, 23, 27-31.

Jensen, S. & G. Sundström : Structures and levels of most chlorobiphenyls in two technical PCB products and in human adipose tissue. Ambio 1974, 3, 70-76.

Johnstone, G.J., D.J. Ecobichon & O. Hutzinger : The influence of pure polychlorinated biphenyl compounds on hepatic function in the rat. Toxicol. Appl. Pharmacol. 1974, 28, 66-81.

Kihlström, J.E., J. Örberg, C. Lundberg & P.O. Danielsson: Post-natal growth in mice sucking milk containing PCB or DDT. Ambio 1974, 3, 231-233.

Kuntzman, R., M. Jacobson, K. Schneidman & A.H. Conney: Similarities between oxidative drug-metabolizing enzymes and steroid hydroxylases in liver microsomes. J. Pharmacol. exp. Therap. 1964, 146, 280-285.

Melvås, B. & I. Brandt : The distribution and metabolism of labelled polychlorinated biphenyls in mice and quails. PCB Conference II National Swedish Environment Protection Board. Publication 4 E, Stockholm, 1973, 87-100.

Örberg, J.: A comparison between some effects of two pure chlorobiphenyls (2,4′5 - trichlorobiphenyl and 2,2′4,4′5,5′- hexachlorobiphenyl) on the hepatic drug metabolizing system in the female mouse. Acta pharmacol. toxicol. 1976, 38, 145-151.

Örberg, J.: Placental and mammary transfer of two PCBs (2,4′5 - TCB and 2,2′4,4′5,5′- HCB) and their effect on the reproductive capacity in mice. Ambio 1977, 5, 278-280.

Örberg, J. & C. Ingvast : Effects of pure chlorobiphenyls (2,4′5 - trichlorobiphenyl or 2,2′4,4′5,5′- hexachlorobiphenyl) on the disappearance of ^{14}C from the blood plasma after intravenous injection of 4 - ^{14}C - progesterone and on the hepatic drug metabolizing system in the female rat. Acta pharmacol. toxicol. 1977, 41, 11-17.

Örberg, J. & J.E. Kihlström : Effects of long-term feeding of polychlorinated biphenyls (PCB, Clophen A 60) on the length of the oestrous cycle and on the frequency of implanted ova in the mouse. Environ. Res. 1973, 6, 176-179.

Platonow, N.S., E.B. Meads, R.M. Liptrap & F. Lotz: Effects of some commercial preparations of polychlorinated biphenyls in growing piglets. Can. J. comp. Med. 1976, 40, 421-428.

Ringer, R.K., R.J. Aulerich & M. Zabik : Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. Proc. Amer. Chem. Soc. Meeting. 1972, 12, 149.

Sanders, O.T. & R.L. Kirkpatrick : Effects of a polychlorinated biphenyl (PCB) on sleeping times, plasma corticoids, and testicular activity of white-footed mice. Environ. Physiol. Biochem. 1975, 5, 308-313.

Sissons, D. & D. Welti: Structural identification of polychlorinated biphenyls in commercial mixtures by gas-liquid chromatography, nuclear magnetic resonance and mass spectrometry. J. Chromatogr. 1971, 60, 15-32.

Street, J.C., F.M. Urry, D.J. Wagstaff & A.D. Blau: Comparative effects of polychlorinated biphenyl and organochlorine pesticides in induction of hepatic microsomal enzymes. Amer. Chem. Soc. 158 th Annu. Meeting, Div. Pesticide Chem., September 1969, Abstract 017.

Vos, J.G., J.H. Koeman, H.L. van der Maas, M.C. ten Noever de Brauw & R.H. de Vos: Identification and toxicological evaluation of chlorinated dibenzofuran and chlorinated naphtalene in two commercial polychlorinated biphenyls. Food Cosmet. Toxicol. 1970, 8, 625-633.

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